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# The Effects of Insect Damage on Nitrogen Fixation by Soybeans and Recovery of Rhizobium Japonicum, Strain 110, From Soybean Nodules.

Robert L. Hutchinson
*Louisiana State University and Agricultural & Mechanical College*

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# THE EFFECTS OF INSECT DAMAGE ON $N_2$ FIXATION BY SOYBEANS

and

# RECOVERY OF *RHIZOBIUM JAPONICUM*, STRAIN 110, FROM SOYBEAN NODULES

A Dissertation

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Robert L. Hutchinson
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ABSTRACT

Greenhouse and field investigations were conducted to study the effects of various types of insect-induced damage to the above or below-ground portion of the soybean [*Glycine max* (L.) Merrill] plant on nodulation and $N_2$ fixation.

In the greenhouse, partial defoliation of soybeans by the soybean looper, *Pseudoplusia includens* (Walker), or by mechanical means, was found to reduce significantly the amount of $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight. These reductions in nitrogenase activity were detected as soon as one day after the leaves had been damaged.

In another greenhouse study, injury to the plant stem inflicted by the southern green stink bug, *Nezara viridula* (Linnaeus), was found to reduce significantly the dry weight of nodules produced on soybean plants. Damage caused by this insect was also reflected in significant reductions in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight.

Greenhouse and field studies indicated that stem girdling injury inflicted on soybean plants by the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), reduced the average nodule number and nodule dry weight per plant. Stem girdling injury was also found to decrease the amount of $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight. The degree to which these parameters were reduced was increased greatly when the girdled plants lodged due to weakening of the stem in the girdled area.

Data collected in extensive field investigations during 1976, 1977 and 1978 indicated that natural populations of bean leaf beetle, *Cerotoma trifurcata* (Forster) and the platystomatid fly, *Rivellia quadrifasciata* (Macquart) larvae were capable of significantly reducing $N_2$ fixation ($C_2H_4$ produced) and yield of soybeans by invading the nodules and consuming the contents. In field studies near Krotz Springs and Port Barre, La., carbofuran and aldicarb were used successfully to control naturally existing soil-borne populations of these predacious larvae. As a result, $N_2$ fixation and yields in the insecticide treated plots were increased by as much as 92 and 29% respectively.

Field tests were conducted during 1976, 1977 and 1978 to determine the percentage recovery of a non-indigenous strain of *Rhizobium japonicum* (Kirchner) Buchanan (strain 110) in nodules when this nodulating bacterium was banded in contact with the seed at rates of $10^4$ and $10^8$ cells per cm of row. The soil chosen for this study contained approximately $10^5$ *R. japonicum* per g of soil. Recovery of serogroup 110 ranged from 3.5 to 9.7% during the three years. Seed inoculation with strain 110 or peat-base inoculant did not increase nodulation, $N_2$ fixation ($C_2H_4$ production), seed yield or percent N in the seeds.

Another purpose of this study was to determine effects of fertilizing nodulating and non-nodulating soybeans with 250 kg/ha of $NH_4NO_3$-N applied in five equal applications. It was hoped that the split applications of N would not adversely affect nodulation or $N_2$ fixation of the nodulating soybean and that yields could be increased above the levels obtained with $N_2$ fixation alone. The results of the study, however, indicated that nodulation and $N_2$ fixation ($C_2H_4$ production) were significantly reduced, and yields were not increased. Seed yield and percent N in the seeds of unfertilized non-nodulating soybeans was significantly lower than for the nodulating soybeans. Application of 250 kg/ha of $NH_4NO_3$-N to non-nodulating soybeans was adequate to produce seed yields that were not significantly different from unfertilized, nodulating soybeans. The contribution of symbiotically fixed $N_2$ to the seeds of the nodulating 'Lee' soybeans was estimated to range from 63.5 to 128.9 kg/ha.

# INTRODUCTION

In recent years the acreage devoted to the growth of soybeans has greatly increased, due largely to an increased demand for oil and protein. As a result, the value of soybeans has increased significantly. Research programs have been very successful in improving insect and weed control practices. Plant breeders have introduced many new cultivars of soybeans that produce higher yields and are more resistant to diseases and pests than the older cultivars.

In spite of advances in the culture of soybeans, yields have remained relatively low compared to most other field crops. In Louisiana average yields have remained below 1700 kg/ha. Many researchers believe that symbiotic $N_2$ fixation is the major factor limiting soybean yields. For this reason much work is being conducted at present to understand better the factors that influence the symbiotic relationship so that steps can be taken to increase $N_2$ fixation.

Most of the research conducted on insect pests of soybeans has been concentrated on damage inflicted on the above-ground portions of the plant such as the leaves, stems and pods and the effects of damage to these plant parts on growth and yield. Very little work has been done to determine the effects of damage to leaves and stems on $N_2$ fixation by soybeans. Even less work has been devoted to studying the effects of nodule-damaging insects on $N_2$ fixation by soybeans and the ability of these plants to compensate for this type of injury.

Greenhouse and field investigations were initiated to study the effects of various types of damage to the above-ground and below-ground portions of the soybean plant on nodulation, $N_2$ fixation and seed yield. Greenhouse investigations included studies on the effects of partial defoliation caused by the soybean looper, *Pseudoplusia includens* (Walker), stem girdling by the three-cornered alfalfa hopper *Spissistilus festinus* (Say) and damage to the plant stem caused by the piercing-sucking proboscis of the southern green stink bug, *Nezara viridula* (Linnaeus) on $N_2$ fixation.

Field studies were conducted to determine the effects of nodule damage caused by larvae of the bean leaf beetle, *Cerotoma trifurcata* (Forster), banded cucumber beetle, *Diabrotica balteata* Le Conte and the platystomatid fly, *Rivellia quadrifasciata* (Macquart) on $N_2$ fixation by soybeans.

Although many soybean growers routinely inoculate soybeans with inoculants presumably containing strains of *Rhizobium japonicum* (Kirchner) Buchanan selected for their superior $N_2$-fixing capabilities, the value of this practice has been questioned repeatedly. In recent years many researchers have demonstrated that seed inoculation is of no value in fields where soybeans have been grown previously. Evidence has been presented indicating that seed inoculation with superior strains of *R. japonicum* is often of little or no value because the number of nodules formed by the added strain is very low. Exceptions to these results have been reported, however, when indigenous populations of *R. japonicum* are very low or where exceptionally large numbers of *R. japonicum* have been applied.

A three year field investigation was conducted to study the effects of soybean seed inoculation with two rates of a non-indigenous strain of

*R. japonicum* on the percent recovery of applied *Rhizobium* in the nodules. Other objectives of the study were to determine effects of the non-indigenous strain of *R. japonicum* on nodulation, $N_2$ fixation, seed yield and seed N content. Nodulating and non-nodulating isolines of soybeans to which no fertilizer N was applied were included in this investigation so that estimates of the contribution of $N_2$ fixation to the seeds of the nodulated soybeans could be made. Nodulating and non-nodulating isolines of soybeans were also fertilized with 250 kg of $NH_4NO_3$-N/ha applied in five equal increments during the growing season so that the effects of N fertilization on nodulation, $N_2$ fixation and seed yield could be studied.

## LITERATURE REVIEW

### I. NITROGEN NUTRITION OF SOYBEANS

According to Brill et al. (1975) "effectively nodulated legumes are the major source of biologically fixed $N_2$ for agricultural production." The N requirement of grain legumes is much higher than for cereals. For example, a 33 bushel per acre soybean crop requires about 200 pounds of N/acre. An equal yield of corn requires the uptake of approximately 50 pounds of N/acre. These differences may be explained by the much higher protein content of soybeans (40-45%) as compared to cereals (approximately 10%) (Brill et al., 1975).

Under normal conditions soybeans, Glycine max (L.) Merrill, obtain most of the N necessary for growth and development from two main sources, soil N and symbiotically fixed $N_2$ (Van Schreven, 1958). The relative contributions from these two sources have been determined by several researchers; the results, however, have been very inconsistent.

Hardy et al. (1968), using the $C_2H_2$ reduction assay, estimated that 74 to 81 kg of $N_2$/ha may be fixed by a soybean crop. According to Hardy and Havelka (1975b), only about 25% of the N accumulated by the plant is contributed by symbiotic $N_2$ fixation during the entire growth cycle of soybeans. It is the belief of these researchers, therefore, that soil N accounts for the majority of the N required by the soybean crop.

The $^{15}N$ and Kjeldahl analyses were employed by Stewart (1966) who reported that 94 kg/ha of N were contributed by symbiotic $N_2$ fixation.

Norman and Krampitz (1945) estimated that in a prairie soil of medium fertility, the contribution of N from symbiotic fixation may account for 25 to 30% of a soybean crop. Weber (1966) used nodulating and non-nodulating soybean isolines to study the relative contributions of soil and symbiotically fixed $N_2$. It was concluded that atmospheric $N_2$ may account for up to 40% of the total in favorable seasons. This was equal to about 78 kg/ha of symbiotically fixed $N_2$. Under unfavorable conditions such as during dry conditions, the contribution of N from the atmosphere was as low as 13% of the total. It was also demonstrated that the contribution from symbiosis was increased to 74% when ground corn cobs were used to partially immobilize the soil N.

According to Nutman (1965) vigorously growing, well-nodulated legumes could supply their own N needs by fixation even when none is abailable from the soil. For this reason N-containing fertilizers are not normally used on soybeans. Hardy and Havelka (1974) stated that N fertilization of soybeans had consistently failed to increase soybean yields because the addition of fertilizer N decreases $N_2$ fixation.

The exact cause for the reduction of $N_2$ fixation resulting from the presence of combined N is unknown. It is generally assumed, however, that any supplemental N absorbed from the soil will divert a certain quantity of photosynthate for increased growth and protein synthesis, thereby reducing the supply of photosynthate to the root nodules. As a result of the reduced supply of photosynthate to the root nodules, $N_2$ fixation is reduced (De Moody et al., 1973). According to Lie (1974), the host plant must enter a period of N hunger before the $N_2$ fixation process becomes active. Under conditions of severe N deficiency, however, research has shown that applications of low levels of fertilizer N could increase growth and nutrient absorbtion more than it decreased $N_2$ fixation (Allos and Bartholomew, 1955).

## II. SYMBIOTIC NITROGEN FIXATION

All organisms that have been proven capable of fixing $N_2$ belong to the kingdom prokaryota. Some of these organisms are capable of fixing $N_2$ only in symbiotic association with higher plants. Agronomically, these organisms are the most important ones (Stewart, 1966).

The most important and widely known of the symbiotic $N_2$ fixing bacteria are those belonging to the genus Rhizobium. These bacteria form root nodules on leguminous plants and fix $N_2$ which is readily available to the host plant. This relationship, known as symbiotic $N_2$ fixation, exists between the soybean plant and the bacterium Rhizobium japonicum (Kirchner) Buchanan. In this relationship the soybean plant is benefited by the $N_2$ which is fixed into an available form by the bacteria, and the bacteria rely on the soybean plant for protection and an energy source.

Molecular $N_2$ is a very stable molecule and is therefore a difficult one to reduce. The first stable intermediate of the fixation process is ammonia ($NH_3$) (Burris, 1942; Bergerson, 1971). The hydrogen ions necessary for the formation of $NH_3$ are believed to be donated by the enzyme hydrogenase (Burris, 1971; Wilson, 1971).

Reduced ferredoxin, a necessary cofactor for symbiotic $N_2$ fixation, has a reduction potential of -0.43 volts and is considered to be the physiological electron donor responsible for reduction of the triple bond of $N_2$ (Burris, 1971; Lehninger, 1970). According to Burris (1971) $Mg^{2+}$ is also a necessary cofactor for $N_2$ fixation.

The phosphate bond energy of ATP has been found to be a necessary component of the $N_2$ fixing system, but its exact function has not been established (Bergerson, 1971; Burris, 1971). According to Burris, though, one function of ATP is to aid in the transfer of electrons from reduced ferredoxin to $N_2$. Four ATP molecules are required for each pair of electrons transferred by nitrogenase (Winter and Burris, 1968).

Gutschick (1978) indicated that 10-12 ATP molecules and three reductants each donating two $H^+$ are used by nitrogenase enzyme to reduce each $N_2$ molecule. He also stated, though, that about 40% of the ATP and reductant are lost in a side reaction that produces $H_2$. In some species two or three ATP's may be recovered from the $H_2$. A total of 12-15 ATP's and 4.2 reductants, therefore, were considered necessary for each $N_2$ molecule reduced (Gutschick, 1978). These values did not take into account the energy input necessary to support the symbiotic relationship such as respiration and growth of nodules. Carbohydrates produced in the leaves of the host plant are translocated to the root nodules of legumes and serve as the energy source necessary for the production of ATP and reductant (Evans and Russell, 1971).

The nitrogenase enzyme is another necessary factor for the $N_2$ fixation reaction to proceed. This enzyme exists as a complex of two proteins, neither of which is capable of functioning alone (Burris, 1971). One is an Fe protein having a molecular weight of about 45,000; the other is an Fe-Mo protein with a molecular weight approaching 150,000 (Lehninger, 1970).

The enzyme system is inactivated by $O_2$, and it is believed that the hemoprotein, leghemoglobin, prevents inactivation of the enzyme by limiting the concentration of $O_2$ in the vicinity of the enzyme (Wilson, 1971; Bergerson, 1971). Leghemoglobin is also believed to carry $O_2$ to the nodule bacteria to be used in cellular respiration (Vest et al., 1973; Bergerson, 1971). This compound is also responsible for the deep pink color within active root nodules (Bergerson, 1963). The formation of leghemoglobin is genetically controlled by the host plant; however, it is not formed in the absence of the symbiotic bacteria (Lehninger, 1970).

Jordan and Garrard (1951) found a close correlation between the leghemoglobin content of root nodules and $N_2$ fixation. As a result of their findings, they concluded that the presence or absence of nodule hemoglobin could be used successfully to classify nodule bacteria into effective and ineffective strains.

Nitrogenase is a very versatile enzyme in that it is capable of reducing $H_3O^+$ and a variety of triple bonded substrates. One example is acetylene ($C_2H_2$) which is reduced to ethylene ($C_2H_4$) in the presence of the enzyme. This reduction coupled with gas chromatographic analysis has been extremely important as a tool for studying $N_2$ fixation (Hardy and Havelka, 1975b).

## III. FACTORS AFFECTING NITROGEN FIXATION

A. Nodulation. Nodulation in soybeans is the process by which *Rhizobium japonicum* bacteria gain entrance into the soybean root tissue and form root nodules. This process marks the beginning of the symbiotic relationship that exists between the two species.

The nodulation of soybeans has been reported as occurring in the root hairs, and it has been noted that nodules may begin to form on the host plant as soon as root hairs are formed (Evans and Russell, 1971; Nutman, 1965). The actual process by which the bacterium gains entrance into the root hair is termed infection (Nutman, 1958).

Preceding the infection of legumes there is normally an increase in numbers of rhizobia in the vicinity of the soybean roots, an occurrence that is apparently due to the excretion of substances from the root into the rhizosphere (Evans and Russell, 1971; Manil, 1958).

The first step of the nodulation process involves a curling of the root hairs in response to the Rhizobium bacteria which secrete indole acetic acid which is thought to be in some way partially responsible for curling of the root hair tips (Nutman, 1965). The mechanism by which the bacterium enters the host root is still open to speculation since several explanations have been proposed (Vest et al., 1973; Allen and Allen, 1940; Nutman, 1965; Evans and Russell, 1971; Nutman, 1958).

When the bacteria enter the root hair, the host produces an infection thread which is continuous with the host cell wall (Evans and Russell, 1971; McCoy, 1932). The bacteria multiply within the infection thread which grows toward the base of the host cell, apparently under the direction of the host cell nucleus (Evans and Russell, 1971). According to Beiberdorf (1938), the length of the infection thread may be 70-80 microns and may take from 18-48 hours to grow from the point of infection to the base of the root hair (Beiberdorf, 1938). There is continuous growth of the infection thread until it reaches one of the few tetraploid cells of the root cortex (Manil, 1958; Evans and Russell, 1971).

The stress of cell enlargement causes the infection thread to rupture, releasing the bacteria into the host cell cytoplasm (Beiberdorf, 1938). The presence of the bacteria induces the tetraploid and adjacent diploid host cells into meristematic activity, thereby forming the root nodule (Evans and Russell, 1971; Vest et al., 1973). The bacteria are disseminated among the host cells during the process of mitosis (Beiberdorf, 1938). Eventually small groups of bacterial cells are enclosed in a membranous envelope (Goodchild and Bergerson, 1966). At this time the bacteria become more elongate and vacuolated and are referred to as bacteroids (Vest et al., 1973). The bacteroid zone of the root nodule is the site of symbiotic $N_2$ fixation.

The nodulation process is influenced to a great extent by the amount of available combined N present in the soil. Tanner and Anderson (1964) found that nitrates in the rhizosphere could severely reduce root hair infection, nodule formation and nodule size.

Weber (1966) revealed that 168 kg/ha of combined N reduced nodule numbers by 33%, fresh weight by 50% and nodule size by 25%. It has also been suggested that supplemental fertilizer N may cause premature nodule decay (Lawn and Brun, 1974).

B. Photosynthate. Lindstrom et al. (1952) were the first workers to establish definitely the dependence of $N_2$ fixation on photosynthesis. At the time, however, they were only able to theorize on the nature of this relationship. Recent research indicates that a substantial amount of energy must be expended in order to reduce $N_2$ to $NH_3$. This energy is provided by the oxidation of photosynthate which is produced in the plant leaves and translocated to the root nodules (Bach et al., 1958).

According to Brill et al. (1975), an adequate supply of photosynthate to the root nodules is a major factor limiting $N_2$ fixation by legumes. A portion of the photosynthate that would be used for synthesis of plant material by most species is utilized by legumes to fix $N_2$. As a result, yields of legumes are normally lower than most cereals and other plants (Nutman, 1976).

The efficiency of $N_2$ fixation has been calculated by Minchen and Pate (1973). They calculated an efficiency of 10% or 18.8 g of glucose consumed per g of $N_2$ fixed. Gutschick (1978) indicated an efficiency of about 12% or a cost of 12.0 g of glucose used per gram of $N_2$ fixed.

Sucrose accounts for the greatest percentage of photosynthate transported by the phloem from the soybean leaf (Bach et al., 1958; Burley, 1961). Small amounts of many other compounds such as serine, glycine, alanine, glutamate, glyceric acid, glucose, raffinose, fructose, malic acid, isocitrate, succinate, aspartate and citrate may also be translocated (Vernon and Aronoff, 1952). It is these products of photosynthesis and respiratory intermediates that provide the energy source necessary to drive the $N_2$ fixation reaction. In addition some of the organic acids are aminated to form amino acids which may be moved into the root. These amino acids may then be translocated throughout the plant (Pate, 1962; Wilson, 1971).

Minchin and Pate (1973) constructed a budget for C and N in the shoot, root and nodules of the garden pea (*Pisum sativum* L.). It was determined that 32% of the C fixed by the shoot was translocated to the root nodules. The percentages of C translocated to the nodules that were used for growth and respiration were 5 and 12% respectively. Twelve percent of the C was returned to the shoot in the form of amino compounds. The roots utilized 42% of the fixed C for growth and respiration. Only 26% of the photosynthetically fixed C was utilized directly by the shoot for dry matter production.

In a maturing soybean plant the seed becomes the primary sink for carbohydrates produced in the leaves. As a result, the soluble carbohydrate level in the stems and roots decreases during pod filling, and $N_2$ fixation is greatly reduced (Hardy et al., 1968; Sloger, 1969; Dunphy and Hanway, 1976; Weil and Ohlrogge, 1975).

Recent research indicates that during pod filling, the reduced supply of carbohydrate to the roots may also result in a decline in N, P, K and S uptake (Lawn and Brun, 1974b; Garcia and Hanway, 1976). To compensate for this occurrence, these nutrients are translocated to the seeds from the leaves and other plant parts. This nutrient depletion, or self-destructive characteristic of soybeans, results in decreased rates of photosynthesis and premature senescence of the leaves. Garcia and Hanway (1976) found that foliar applications of N, P, K and S during the seed-filling stage could significantly increase soybean yields. They concluded that the observed yield increases were a result of decreasing the nutrient depletion from the leaves during the seed-filling period. This allowed the leaves to continue producing carbohydrates for a longer period of time.

Hardy and Havelka (1975a) suggested that this self-destructive period could be eliminated if adequate photosynthate could be produced by the soybean plant during the seed-filling period. The resulting increase in photosynthate could be utilized by the plant to increase yields by increasing $N_2$ fixation and delaying senescence (Hardy, 1977).

More research evidence on the importance of photosynthate production to $N_2$ fixation was cited by Sanders and Brown (1976) who discussed the effects of varying the shoot; root ratios of soybean seedlings by grafting. Increasing the number of shoots per root resulted in an increase in total leaf area, number of leaves per plant, seed yield, number of seeds per plant and nutrient uptake by the roots. However, in the same study it was deduced that increasing the number of roots per shoot resulted in a greater increase in seed yield, growth and nutrient uptake than occurred by increasing the number of shoots per root. This indicated that the photosynthetic portion of the plant is very important for maximum soybean yields. Nonetheless, the root system plays an equally important role.

Hardy and Havelka (1974) reported that the rate of $N_2$ fixation is low during the early stages of the growth of soybeans and increases as the plant approaches maturity. According to Hardy and Havelka (1975b), about 10% of the $N_2$ is fixed by field-grown soybeans during the vegetative growth stage, and 90% is fixed during the reproductive stages. According to these researchers, the rate of $N_2$ fixation increased by about 6% per day after the plants were about 30 days of age. Fixation was reported to be at its maximum rate at about 80 days. It was also noted that during pod filling, $N_2$ fixation is decreased because the seeds are competing with the nodules for available photosynthate.

Research conducted by Hardy and Havelka (1974) demonstrated that increasing a plant's photosynthetic rate can result in a tremendous increase in $N_2$ fixation. Increasing the $CO_2$ concentration from 350 ppm to 1200 ppm in the soybean leaf canopy increased $N_2$ fixation approximately fourfold. The increased $N_2$ fixation was attributed to increased nodule mass and increased fixation per gram of nodule.

Other studies by Hardy et al. (1968) indicated that in field-grown soybeans $C_2H_2$-reducing activity ($N_2$ fixation) was greatest between noon and 8 P. M. and reached a minimum level between midnight and 8 A. M. From these results it was theorized that this decline was due to the depletion of photosynthate, while the residual activity was perhaps due to utilization of storage products.

Although adequate photosynthate is necessary for optimum $N_2$ fixation, several authors have reported that defoliation of soybeans during the vegetative stages of growth has little effect on seed yields. Weber (1955) demonstrated that until full bloom, defoliation of 50 to 100% gave a maximum of 20% yield reduction. Jackson (1967) found that 50-75% defoliation before blooming could be tolerated without serious loss in yield.

Similar results were cited by Turnipseed (1972) who reported defoliation of 17 to 33% during bloom did not decrease soybean yields in ten tests. Defoliation of 17 to 33% allowed better light penetration to the lower leaves, resulting in compensation due to an increased rate of photosynthesis by these leaves. In the same experiment, defoliation of 50 to 67% at the pod-setting stage resulted in a significant reduction in seed yield. These data illustrate the increased demand for photosynthate during the pod-filling stage of development.

C. Special Mineral Requirements. The mineral requirements of non-nodulated legumes is essentially the same as for non-leguminous plants. The unique relationship that exists between *Rhizobium* species and legumes, however, necessitates slightly different mineral requirements.

All higher plants require Mo for the reduction of nitrates by the enzyme nitrate reductase. Legumes, however, require a much greater amount of Mo because it is also an important constituent of nitrogenase (Evans and Russell, 1971). In legumes, therefore, Mo serves a dual function in N metabolism; as a result, much larger quantities are needed to satisfy the plants' requirements (Parker and Harris, 1977). Due to the role that Mo plays in the nutrition of legumes, the characteristic symptoms of Mo deficiency are identical to N deficiency symptoms (Hewitt, 1958).

Studies by several researchers have demonstrated that small amounts of Co are essential to symbiotic $N_2$ fixation by legumes (Ahmed and Evans, 1961; Delwiche et al., 1961; Hallsworth et al., 1960). In each instance the reported symptoms of Co deficiency were identical to N deficiency.

Calcium is another essential nutrient element for all plants; however, legumes require a growth medium that is higher in Ca and alkalinity than most other plants. Adequate Ca in the soil is necessary for successful infection of legume roots by *Rhizobium* (Lowther and Loneragan, 1968).

Although Fe is essential in small amounts to all plants, an additional amount is necessary for $N_2$ fixation by nodulated legumes. This can be explained by the requirements for Fe by the nitrogenase enzyme and the leghemoglobin pigment, both of which are essential to $N_2$ fixation (Evans and Russell, 1971).

Copper also is essential to both legumes and non-legumes if they are to achieve normal growth and development. Hallsworth et al. (1960) suggest that an additional amount of this element is necessary for $N_2$ fixation in legumes. They stated that Cu may play an important role in leghemoglobin synthesis.

D. Soil Moisture. Research has revealed that soybean plants subjected to moisture stress undergo a reduction in $N_2$ fixation. According to Sprent (1972), the optimum moisture conditions for symbiotic fixation are approximately field capacity. It was noted that under greenhouse and field conditions, significant reductions in $N_2$ fixing activity usually coincided with wilting of the lower leaves.

Sprent (1972) also discovered that plants subjected to moderate moisture stress were able to recover all of their $N_2$ fixing activity within three hours after watering. The $N_2$ fixing activity of severely stressed plants (all adult leaves beyond permanent wilting point) was found to increase slowly after watering, but recovery was only partial.

It was also reported that nodule activity decreased when the soil moisture content exceeded field capacity. This occurrence was attributed to soil waterlogging and subsequent $O_2$ deficiency.

## IV. SEED INOCULATION

Several researchers have demonstrated the beneficial effects of inoculating soybean seeds with Rhizobium japonicum before planting. These beneficial results, however, have been limited almost exclusively to soils where soybeans had not been grown previously (Pendleton and Hartwig, 1973; Abel and Erdman, 1964; Caldwell and Vest, 1970; Ham et al., 1971). For this reason, inoculation of soybeans is usually not recommended except on newly cleared land or fields where soybeans will be grown for the first time (Pendleton and Hartwig, 1973).

Research conducted by Erdman and Wilkins (1928) indicated that certain strains of rhizobia are required to produce maximum nodulation of a given soybean variety.

According to Sloger et al. (1974) the tap root nodules are the major sites of $N_2$ fixation during vegetative growth, however, these nodules senesced before the smaller nodules located on the lateral roots. Stewart (1966) stated that "ineffective" root nodules which are restricted to the lateral roots tend to be small, white colored and fix little or no $N_2$. On the other hand, "effective" nodules fix much more $N_2$ and are larger with pink interiors due to an abundant leghemoglobin content.

Recent research indicates that in most instances _Rhizobium japonicum_ strains added to the soil or to the seed prior to planting do not compete very well with naturalized strains for nodule sites. Johnson et al. (1965) reported that the average recovery of applied strains was only 5% when applied at the standard inoculation rate. Caldwell and Vest (1970) were able to recover only 5-10% of applied _Rhizobium_ from soils that contained native rhizobia. In this experiment a peat-base inoculum was applied to the seed at about 25 times the recommended rate or about 250,000 viable bacteria per seed. Ham et al. (1971) reported recovery of 0-17% depending on the strain of inoculant used and the location of the experiment.

In soils that do not contain naturalized populations of rhizobia, several researchers have shown that seed inoculation with _Rhizobium japonicum_ increases seed yields, seed protein content, nodulation, fresh plant weight and oil content of the seeds (Abel and Erdman, 1964; Caldwell and Vest, 1970). It has also been established that in soils containing no native rhizobia, inoculation with different strains of rhizobia often results in significant yield differences. These differences were not found when the strains were applied in soils already containing naturalized rhizobia (Caldwell and Vest, 1970; Abel and Erdman, 1964). Caldwell (1969) found that in soils containing no native rhizobia strains differed in competitive ability when applied simultaneously as a mixture of strains.

Sloger (1969) indicated that some strains of rhizobia were capable of fixing more $N_2$ per plant and per gram of fresh nodule weight than other strains. On the other hand, some strains of rhizobia may actually enter a parasitic relationship with the host plant (Van Schreven, 1958). It was the opinion of Johnson et al. (1965) that inoculating soybeans with superior strains of *Rhizobium japonicum* while improving inoculation procedures may be the simplest and most economical methods of increasing soybean yields.

Considerable evidence has recently been presented indicating that various environmental factors greatly influence the distribution of rhizobia in soils and soybean root nodules. According to Damirgi et al. (1967) this distribution of strains varies greatly with soil type. It was also revealed that soil pH had an influence on which strains were present. Date of planting and stage of plant development when nodules were sampled also influenced the strain distribution in soybean nodules (Caldwell and Weber, 1970).

Weber and Miller (1972) found that soil temperature also has an effect on distribution of *Rhizobium* strains in soybean nodules. Methods of introducing effective *Rhizobium japonicum* symbionts to soybeans under various environmental conditions is seen as a method of increasing the usefulness of this important crop (Delwiche, 1978).

Investigative research has revealed that there is considerable variation in the effectiveness of strains of rhizobia on different soybean cultivars. Vorhees (1915) reported that in plots with several varieties of soybeans that were inoculated with *Rhizobium japonicum*, all of the varieties were well nodulated except one. As a result of these data, he theorized that different varieties of a legume vary in their resistance to association with symbiotic bacteria. Similar results were reported by Erdman and Wilkins (1928). They stated that pure cultures of _Rhizobium japonicum_ isolated from one variety failed to nodulate other varieties as effectively. Later work by Erdman (1944) showed that native strains of rhizobia were usually more "effective" at inducing nodulation in soybeans adapted to that region than strains introduced from a different location. As a result of these findings, it seems reasonable to conclude that environmental factors and host plant cultivar may influence nodulation and $N_2$ fixation just as different strains of rhizobia affect these processes (Wilson, 1940; Van Schreven, 1958).

V. THE BEAN LEAF BEETLE

The bean leaf beetle, _Cerotoma trifurcata_ (Forster), is a member of the order Coleoptera, family Chrysomelidae. This insect is abundant in the southeastern United States and ranges as far north as Kansas, Minnesota and Canada and may be found as far west as New Mexico. The bean leaf beetle attacks beans, peas, cowpeas, soybeans, and several other plants. The adults cause injury to the host plant by feeding on the leaves, stems and occasionally on the flowers and pods (Pears and Davidson, 1956; Metcalf and Flint, 1951; Turnipseed, 1973). Heavy infestations of this insect often cause severe damage to young stands of soybeans. When older plants are attacked and defoliated, losses in yield may be considerable. Both the adult and larvae of the bean leaf beetle have been implicated in the transmission of several soybean diseases including bean pod mottle, cowpea mosaic and southern bean mosaic virus (Metcalf, 1951; Turnipseed, 1973;

Walters, 1964). The larvae of the bean leaf beetle are responsible for feeding on the roots, root hairs and root nodules of soybeans and several other species of legumes. When attacking root nodules, the larvae normally cut a small round hole in the nodule and proceed to devour the entire contents of the nodule, leaving only an empty shell (McConnell, 1915; Eastman, 1976).

Jackson (1967) reported that first generation larvae of the bean leaf beetle destroyed most of the nodules of soybeans in some fields in northern Missouri. He also speculated that this nodule damage was responsible for N deficiency in the affected plants. To his knowledge, however, no literature exists on the specific effects of bean leaf beetle larvae damage on $N_2$ fixation in soybeans. He suggested that there was need for further studies.

## VI. THREE-CORNERED ALFALFA HOPPER

The range of the three-cornered alfalfa hopper (*Spissistilus festinus*) (Say) is confined to the southern and some midwestern states (Turnipseed, 1973). This insect causes damage to soybean plants by injecting its needle-like proboscis into the stem of the plants causing girdling of the stems by encircling them with feeding punctures. As a result, the stems are weakened and are often broken over by winds late in the season (Caviness and Miner, 1962).

According to research by Caviness and Miner (1962) in which they simulated three-cornered alfalfa hopper damage, stand reduction of 45% two weeks before blooming failed to cause a significant decrease in yield. The same authors also reported that if a 45% stand reduction occurred two weeks after blooming, only a 15% decrease in yield was produced. The data also indicated that 30% stand reductions occurring two weeks before and during full bloom caused no significant decrease in yield. Stand reductions of 15% at two weeks before, two weeks after and during full bloom, failed to significantly reduce soybean yields. These data indicated that soybeans are capable of tolerating a considerable amount of damage by three-cornered alfalfa hoppers without experiencing decreases in yield.

Other work by Tugwell and Miner (1967) indicated that natural girdling of 68% of the soybean stems by the insect also failed to decrease soybean yields. These authors did, however, suggest that further research is necessary to adequately determine the economic injury levels of this soybean pest. At present no work has been conducted to determine the effect that stem girdling has on $N_2$ fixation in soybeans.

## VII. THE SOYBEAN LOOPER

The soybean looper, _Pseudoplusia includens_ (Walker) is a lepidopterous larvae which often causes severe losses to soybeans due to defoliation, particularly in states south of a line between Arkansas and North Carolina (Turnipseed, 1973). According to Turnipseed (1973), the soybean looper normally reaches peak infestation levels in late August or September. This insect will eat large holes in soybean leaves, and heavy infestations may completely defoliate plants in only a few days. Several researchers have determined the yield losses that may accompany defoliation of soybeans by this insect and others that defoliate soybean plants (Turnipseed, 1972; Weber, 1955; Begum and Eden, 1965). These yield losses would be directly related to loss of photosynthetic leaf area as discussed in Part B--Photosynthate.

## VIII. THE PLATYSTOMATID FLY

The platystomatid fly, Rivellia quadrifasciata (Macquart) is a dipteran belonging to the family Platystomatidae. The species is most common in the eastern United States, but specimens have been collected from Alabama to Quebec and as far west as Montana (Namba, 1956). At present, very little attention has been devoted to this insect insofar as linking it to soybean damage. Several workers, however, have reported that species of Rivellia have caused 50 to 70% damage to nodules of other legumes (Seegar and Maldagne, 1960; Diatloff, 1965). Both authors made inference to the probability that $N_2$ fixation is considerably reduced.

Eastman (1976) found large numbers of the larvae of Rivellia quadrifasciata in soil samples and inside soybean root nodules in soil samples taken from a small-plot field test. As a result of her findings, Eastman (1976) stated that "damage to soybean nodules in many cases can no longer be attributed solely to bean leaf beetle larvae because as yet differences in nodules damaged by fly or beetle larvae cannot be distinguished." Damage to nodules of soybeans by a complex of bean leaf beetle and Rivellia larvae was found to be 45% in this small-plot field test. Eastman (1976) indicated that further work is necessary to determine the effects of such nodule damage on $N_2$ fixation, nodulation, plant growth and yield.

## IX. STINK BUGS

According to Turnipseed (1973), the three most important members of the stink bug complex on soybeans are the green stink bug, Acrosternum hilare (Say); the southern green stink bug, Nezara viridula (Linnaeus); and the brown stink bug, Euschistus servus (Say).

The southern green stink bug is found only in the Gulf Coast region and may feed on many plants including legumes (Pears and Davidson, 1956). Green and brown stink bugs are common in the South and are present as far north as Missouri and some midwestern states (Turnipseed, 1973).

Stinkbug damage to soybeans results from nymphs and adults sucking sap from pods, buds, blossoms and seeds. When stink bugs remove the liquid contents from developing seeds, seeds are shriveled, and pods may be aborted (Pears and Davidson, 1956; Turnipseed, 1973). If high infestations of stink bugs are not controlled, complete loss of yield may result. Lower infestations of these insects may damage a sizeable percentage of the seed and thereby reduce seed quality.

## X. SEROLOGICAL METHOD OF STRAIN IDENTIFICATION

It is often desirable to determine the strains of Rhizobium japonicum that occur in soybean root nodules and to study the competitiveness of these strains in inducing nodulation of the host plant. The classification of rhizobia is normally accomplished through the use of serological tests. The test most frequently used at present is the agglutination test. This test is based on the fact that bacteria (antigens) agglutinate under certain conditions when mixed with the appropriate antibodies. Rabbits are normally used to produce the antisera used in the agglutination test. The animal is injected with a specific bacterium or strain of bacterium which induces the formation of antibodies in the blood serum. After an appropriate number of injections with the desired antigen, the rabbit is bled, and the serum is separated and preserved. The serum is diluted and added to serological tubes, and the antigen suspension is added. The temperature and time of incubation varies according to the nature of the bacteria or antigen being used. After the desired time has elapsed, each tube is observed to determine if agglutination has occurred. If agglutination occurs, then the test bacteria belong to the same serological group as the bacteria that were used to produce the antiserum (Carpenter, 1965).

According to Vest et al. (1973) this method was first used to classify Rhizobium japonicum by Stevens in 1923. He classified rhizobia by dividing 8 strains into 4 distinct serological groups. Wright (1925) found that the serological reactions of 8 strains of Rhizobium japonicum remained constant regardless of the medium on which they were grown and after passage through the soybean plant.

Twenty-eight strains of Rhizobium japonicum from the USDA Beltsville Culture Collection were tested for agglutination with 28 antisera derived from these strains. A total of 17 serological groups (serogroups) were found. A serogroup consisted of 2 or more strains that resulted in positive agglutination reactions when combined with the same antiserum. Within serogroups one or more serological type was differentiated on the basis of agglutinin absorption (Means et al., 1964).

Means et al. (1964) found that antigens from bacteroids of nodules produced agglutination reactions identical to reactions of culture antigens for 15 of 17 strains investigated. As a result of these findings, a fast and simple agglutination test was developed in which crushed nodule suspensions are used as the bacterial antigen source. When tested against the appropriate antisera, the bacterial antigens may be used to determine the serogroups or strains of rhizobia within any root nodule (Means et al., 1964).

# MATERIALS AND METHODS

## I. GREENHOUSE STUDIES ON THE EFFECTS OF INSECT DAMAGE ON NODULATION AND $N_2$ FIXATION BY SOYBEANS

Experiments were conducted in the greenhouse so that the effects of various types of insect damage to the soybean, Glycine max (L.) Merrill, $N_2$-fixing system could be studied under controlled conditions. In each experiment the plants received a specific type of injury after which the $C_2H_2$ reduction assay, as described by Hardy et al. (1968) was performed to determine what, if any, effects this damage had on $N_2$ fixation ($C_2H_4$ production). Several other factors closely related to $N_2$ fixation such as weight and number of nodules per plant were also studied.

Insect damage experiments conducted in the greenhouse employed the soybean looper, Pseudoplusia includens (Walker), the three-cornered alfalfa hopper, Spissistilus festinus (Say), and the southern green stink bug, Nezara viridula (Linnaeus). Several types of simulated insect defoliation were compared to actual insect defoliation in order to determine if these types of damage induced similar effects in soybeans.

### A. Soybean Looper Defoliation Tests

Soybean seeds (cultivar 'Bossier') were inoculated with Nitragin® peat-base soybean inoculant and planted in 7.6-1 polyethylene pots in the greenhouse on September 23, 1976. Each pot contained 5 kg of air-dried Convent soil from St. Gabriel, La. Some of the properties of this soil, as determined by the LSU soil testing laboratory, are listed in Table 1. Five seeds were planted in each of 38 pots, and 10 days after planting the plants were thinned to three seedlings per pot.

In addition to the natural illumination, the plants were grown under fluorescent lights which were automatically turned on at 6:00 A.M. and off at 8:00 P.M. in order to prevent flowering.

On October 28, 1976, four soybean loopers were placed on the leaves of each plant in 19 of the 38 pots. The plants in the remaining 19 pots were left undamaged to serve as controls. Each pot containing three plants represented one experimental unit. The experiment was set up in a completely randomized design.

Table 1. Some properties of the Convent sandy loam soil used for greenhouse pot experiments

| Convent Sandy Loam | |
|---|---|
| Soil reaction (pH) | 7.8 |
| Organic matter | 0.13% |
| Extractable P | 162 ppm |
| Extractable K | 32 ppm |
| Extractable Ca | 1335 ppm |
| Extractable Mg | 130 ppm |

The soybean loopers were allowed to feed on the plants until November 10, 1976 (14 days). At this time all of the plants were harvested by cutting each plant at the soil level and excavating the root systems very carefully. The stage of development of the plants was V5 (Fehr et al., 1971). The total leaf area for the plants in each replication was determined using a Li-Cor® electronic area meter. These values were divided by three to obtain the average leaf area per plant. The $C_2H_2$ reduction assay was performed on the three nodulated root systems of each replication to estimate the $N_2$ fixed ($C_2H_4$ produced) by the nodules.

This procedure, which was used for both field and greenhouse experiments, is summarized as follows:

Intact nodulated root systems of soybeans were carefully excavated from the soil and most of the adhering soil was removed by gently shaking or crumbling the soil away from the roots. The entire root system was then carefully washed in tap water so as not to detach the nodules.

The entire root systems were then placed in a 240 ml (½ pint) canning jar, and the lid was screwed tightly onto the jar. A specific volume of air was removed from the jar by inserting a hypodermic needle and syringe through a rubber septum fitted securely in the lid. In all field and greenhouse experiments in 1976 and 1977, 10.0 cc of air was removed; in the 1978 field experiments 25.0 cc of air was removed. The air that was removed from the jars was immediately replaced with an equal volume of $C_2H_2$. After exactly one hour, 10.0 cc of the gas mixture within the jar was removed with a syringe and injected into a 10 ml evacuated tube (Vacutainer®).

Subsamples of gas from the Vacutainer® were injected into a Perkin Elmer® Model 3920 gas chromatograph equipped with a dual hydrogen flame detector system. A single channel recorder connected to the gas chromatograph recorded the peak heights of $C_2H_4$ formed by each sample.

Standard curves of peak height on the recorder versus moles of $C_2H_4$ were prepared for each experiment in order to determine the amount of $C_2H_4$ in the sample. The $C_2H_4$ standard gas mixture was prepared by injecting 100 $\mu$l of pure $C_2H_4$ into a 183 ml bottle sealed with a rubber serum cap.

Several volumes, from 100 to 500 $\mu$l, of the $C_2H_4$ standard were injected into the gas chromatograph, and the peak heights for the known volumes of $C_2H_4$ were determined. A standard curve of peak height versus moles of $C_2H_4$ was then prepared. The standard curve and a series of mathematical calculations was used to convert $C_2H_4$ peak height from each sample to moles of $C_2H_4$ formed by the root nodules of the plants.

Nitrogenase is capable of reducing a number of substrates including $N_2$ and $C_2H_2$, a direct relationship exists between $N_2$ fixation and the reduction of $C_2H_2$ to $C_2H_4$. Therefore the production of $C_2H_4$ was used as an estimator of nitrogenase activity.

The root nodules were counted as they were removed from the root systems and the total number was divided by three to obtain the average number of nodules per plant for each sample. The dry weight of nodules per sample was determined by drying the nodules for three days at 60°C. These values were also divided by three to obtain the average dry weight of nodules per plant in each sample.

The total quantity of $C_2H_4$ in micro-moles ($\mu M$) produced per plant by the reduction of $C_2H_2$ was divided by the number of plants per sample, number of nodules per sample and dry weight of nodules per sample to obtain the various parameters of nitrogenase activity. In some studies $\mu M$ of $C_2H_4$ was also divided by $cm^2$ of leaf area. These parameters are summarized below for clarity:

1. $\mu M$ of $C_2H_4$ per plant,
2. $\mu M$ of $C_2H_4$ per nodule,
3. $\mu M$ of $C_2H_4$ per g of dry nodule,
4. $\mu M$ of $C_2H_4$ per $cm^2$ of leaf area.

A second experiment was conducted to study the effects of soybean loopers on nodulation and $N_2$ fixation ($C_2H_4$ production) by soybeans.

'Bossier' soybean seeds were inoculated with Nitragin® peat-base soybean inoculant and planted in 7.6-l plastic pots in the greenhouse on November 5, 1976. Each pot contained 5 kg of air-dried Convent soil. A total of 20 pots were used and five seeds were planted in each pot. After 10 days the seedlings were thinned to three plants per pot.

On December 10, 1976, five weeks after planting, four soybean loopers were placed on the leaves of each plant in 10 of the pots. The remaining 10 pots were left undamaged to serve as controls; therefore the experiment consisted of ten replications of damaged and control plants in a completely randomized design.

The soybean loopers were allowed to feed on the plants until December 20, 1976 (10 days), at which time all of the control and looper-damaged plants were harvested. The stage of development of the plants was V5. The three root systems from each pot were combined, and the $C_2H_2$ reduction assay was performed. The total leaf area for the plant tops in each pot was determined with the area meter. The nodules were removed from the root system of each plant, and the number and dry weight of nodules per plant was determined. The parameters for nitrogenase activity were determined for each sample as described previously.

B. <u>Simulated Insect Defoliation Versus Soybean Looper Defoliation Tests</u>.

Five experiments were conducted in the greenhouse to evaluate two types of mechanical defoliation as a means of simulating defoliation of soybeans caused by insects such as the soybean looper.

In the first experiment, 'Bossier' soybeans were inoculated with Nitragin® peat-base inoculant and planted in 7.6 l polyethylene pots in the greenhouse on December 3, 1976. Five seeds were planted in each pot, with each of the pots containing 5 kg of air-dried Convent soil. A total of 30 pots was used in the experiment. Ten days after planting, the seedlings were thinned to two plants per pot.

The experiment consisted of ten replications of three treatments in a completely randomized design. The treatments were as follows:

Treatment 1. Plants were left undamaged to serve as controls.

Treatment 2. A paper punch was used to remove circular pieces of leaf tissue from each leaflet on January 21, 1977 in order to approximate 30% defoliation (Punched).

Treatment 3. On January 21, 1977, one leaflet was removed from each trifoliate leaf to give approximately 30% defoliation (Clipped).

On January 27, 1977, all plants were harvested and leaf area, number of nodules, dry weight of nodules and nitrogenase activity were determined for each sample. At harvest the stage of development of the plants was V6.

On January 21, 1977, 28 pots were planted with 'Bossier' soybeans at the rate of five seeds per pot. The seeds had been inoculated with Nitragin® peat-base inoculant. Each 7.6 l polyethylene pot contained 5 kg of air-dried Convent soil. After 10 days the seedlings were thinned to two plants per pot. The experiment consisted of seven replications of four treatments in a completely randomized design. The treatments were as follows:

Treatment 1. All plants were left undamaged to serve as controls.

Treatment 2. A paper punch was used to remove sections of leaf tissue from each leaflet on March 7, 1977, to approximate 30% defoliation (Punched).

Treatment 3. One leaflet was removed from each trifoliate leaf on March 7, 1977, in order to achieve approximately 30% defoliation (Clipped).

Treatment 4. Eight soybean loopers were placed on each plant on March 7, 1977 (Looper damaged).

On March 15, 1977, all plants were harvested and the leaf area, nodule number, nodule dry weight and nitrogenase activity were determined for each plant. The stage of development of the plants at harvest was V6.

A third experiment was conducted in the greenhouse to study the effects of simulated insect defoliation on $N_2$ fixation ($C_2H_4$ production) by soybeans. 'Bossier' soybeans were inoculated with Nitragin® peat-base inoculant and planted at the rate of five seeds per 7.6-l pot on February 30, 1977. Each pot contained 5 kg of air-dried Convent soil. Two weeks after planting the seedlings were thinned to two plants per pot. In all, 40 pots were used in the experiment, which consisted of 10 replications of four treatments in a completely randomized design. Each pot containing two plants represented one experimental unit. The four treatments included in the experiment were as follows:

Treatment 1. All plants were left undamaged to serve as controls.

Treatment 2. A paper punch was used to remove circular sections of leaf tissue from each leaflet on April 18, 1977 (Punched).

Treatment 3. One leaflet was removed from each trifoliate leaf on each plant on April 18, 1977 (Clipped).

Treatment 4. Eight soybean loopers were placed on each plant on April 18, 1977 (Loopers).

On April 21, 1977, all of the plants were harvested and the $C_2H_2$ reduction assay was performed. The stage of development of the plants was V6. The total leaf area, nodule number, dry nodule weight and nitrogenase activity were determined for each plant.

A fourth experiment was conducted to study the effects of simulated insect defoliation and soybean looper defoliation on $N_2$ fixation by 'Bossier' soybeans. Thirty 7.6 l plastic pots filled with 5 kg of Convent soil were planted with five seeds per pot on May 10, 1977. The seeds were again inoculated with Nitragin® peat-base inoculant. The seedlings were thinned to one plant per pot 10 days after planting. A total of 30 pots were used in the experiment which consisted of 10 replications of three treatments in a completely randomized design. The treatments included in this study were as follows:

Treatment 1. All of the plants were left undamaged to serve as controls.

Treatment 2. One leaflet was removed from each trifoliate leaf on June 21, 1977 (Clipped).

Treatment 3. Ten soybean loopers were placed on the leaves of each plant on June 16, 1977. On June 21, 1977, the loopers were removed from each plant by hand (Looper damaged).

On June 27, 1977, all plants were harvested and the $C_2H_2$ reduction assay was performed. Nodule number, nodule dry weight per plant and the usual parameters for nitrogenase activity were determined for each plant. At harvest the stage of development of the plants was V6.

A final experiment was conducted to study the effects of soybean looper defoliation, simulated insect damage and time of defoliation on $N_2$ fixation by soybeans. On February 23, 1977, 'Bossier' soybean seeds were inoculated with Nitragin® peat-base inoculant and planted in 7.6-l polyethylene pots containing 5 kg of air-dried Convent soil. Two weeks after planting the seedlings were thinned to two plants per pot. The experiment consisted of nine treatments with six replications per treatment in a completely randomized design. Each experimental unit consisted of the two plants within each pot. The treatments included in the experiment were as follows:

Treatment 1. On March 31, 1977, six soybean loopers were placed on the leaves of each plant and allowed to feed for five days (Loopers - 5 days).

Treatment 2. Same as Treatment 1 except the soybean loopers were placed on the plants on April 2, 1977, and allowed to feed for 3 days (Loopers - 3 days).

Treatment 3. Same as Treatment 1 except the soybean loopers were placed on the plants on April 4, 1977, and allowed to feed for one day (Loopers - 1 day).

Treatment 4. On March 31, 1977, a paper punch was used to remove sections of leaf tissue from each leaflet to approximate 30% defoliation (Punched - 5 days).

Treatment 5. Same as Treatment 4 except defoliation was performed on April 2, 1977 (Punched - 3 days).

Treatment 6. Same as Treatment 4 except defoliation was performed on April 4, 1977 (Punched - 1 day).

Treatment 7. On April 4, 1977, one leaflet was removed from each trifoliate leaf to approximate 30% defoliation (Clipped 1/3 - 1 day).

Treatment 8. Same as Treatment 7 except two leaflets were removed from each leaf to approximate 70% defoliation (Clipped 2/3 - 1 day).

Treatment 9. The plants in the six remaining pots were left undamaged to serve as controls.

On April 5, 1977, all of the plants were harvested, and the $C_2H_2$ reduction assay was performed. The stage of development of the plants was V5. As in the previous experiments, leaf area, nodule number, nodule dry weight and nitrogenase activity were determined for each plant.

C. Three-Cornered Alfalfa Hopper Tests.

Two greenhouse experiments were conducted to study the effects of stem girdling caused by the three-cornered alfalfa hopper on $N_2$ fixation ($C_2H_4$ production) and nodulation of soybeans.

As in previous experiments 'Bossier' soybean seeds were inoculated with Nitragin® peat-base inoculant and planted in 7.6 l polyethylene pots containing 5 kg of Convent soil. Five seeds were planted in each pot on June 17, 1977, and after 10 days the seedlings were thinned to one plant per pot. The experiment consisted of 10 replications of damaged and undamaged plants in a completely randomized design. Each damaged plant was subjected to continuous feeding by 10 adult three-cornered alfalfa hoppers from July 1, 1977 to July 22, 1977 (21 days).

Small cages made of waxed paper cups with cheesecloth covers were used to contain the insects on the damaged plants. These cages extended from 1 cm below the soil line to about 2 cm below the first trifoliate leaves.

On July 22, 1977, the cages were removed and the $C_2H_2$ reduction assay was performed immediately. The stage of development of the plants was V5. The number and dry weight of nodules per plant and nitrogenase activity were determined for each sample.

The experiment was repeated to confirm the results of the first study. The methods used were the same as for the preceding study. 'Bossier' soybeans which were planted on August 28, 1977, were damaged by three-cornered alfalfa hoppers from October 20, 1977 until November 11, 1977, a period of 22 days. This experiment consisted of 15 replications of damaged and control plants.

On November 11, 1977, all plants were harvested and the same parameters were determined as in the previous experiment. In addition, the fresh weight of each plant top was determined. The stage of development of the plants at harvest was V6.

D. <u>Stink Bug Test</u>

A final greenhouse experiment was conducted to determine if southern green stink bugs could decrease $N_2$ fixation by soybeans by sucking photosynthate from the plant stem. 'Bossier' soybeans were inoculated with Nitragin® peat-base inoculant and planted on November 14, 1977, in 7.6 l pots filled with 5 kg of Convent soil. Five seeds were planted in each pot, but the seedlings were thinned to one plant per pot two weeks after planting. The experiment consisted of 30 plants damaged by stink bugs and another 30 plants that served as controls.

On December 6, 1977, small cages made of waxed paper cups with cheesecloth covers were placed around 30 of the plants. The cages extended from about 1 cm below the soil's surface to approximately

2 cm below the first trifoliate leaf. Three stink bugs which were in the fourth instar stage and two stink bugs in the fifth instar stage were introduced into each cage. One week later, on December 13, 1977, the same number of stink bugs was again added to half of the cages in order to increase the feeding damage. Therefore, the stink bug-damaged plants were divided into two levels of damage (Levels 1 and 2, respectively).

On December 15, 1977, all the plants were harvested, and the $C_2H_2$ reduction assay was performed. The stage of development of the plants was V4. The usual parameters for nitrogenase activity, nodule number and nodule weight were determined for each plant.

## II. FIELD STUDIES ON THE EFFECTS OF INSECT DAMAGE ON $N_2$ FIXATION BY SOYBEANS

Field investigations were conducted at various locations in Louisiana from 1976-78 so that the effects of several types of insect damage could be studied under natural conditions. The types of insect damage that were studied included stem girdling caused by the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), and nodule damage caused by larvae of the bean leaf beetle, *Cerotoma trifurcata* (Forster), banded cucumber beetle, *Diabrotica balteata* LeConte, or the platystomatid fly, *Rivellia quadrifasciata* (Macquart). In each experiment the $C_2H_2$ reduction assay was used to estimate nitrogenase activity of damaged plants and undamaged control plants. Other factors closely related to $N_2$ fixation such as nodule number and nodule dry weight per plant were also studied. In some studies, leaf area and seed yield of the plants were determined so that the effects of insect damage on these important parameters could be determined.

A. <u>Three-Cornered Alfalfa Hopper</u>

A field of 'Bragg' soybeans was selected for an experiment to study the effects of three-cornered alfalfa hopper damage on field-grown soybeans. These soybeans had been planted on June 15, 1977, near Port Barre, La. The plants within the field had received moderate to heavy girdling damage by this insect during the plants' early growth stages and a high percentage of the plants had lodged due to weakening in the girdled area of the stem. For the purpose of this study, damage caused by this insect was divided into the following three groups:

<u>Group a.</u> Control plants that had not been visibly damaged by three-cornered alfalfa hoppers.

<u>Group b.</u> Plants that had been completely girdled but had not lodged.

<u>Group c.</u> Plants that had been completely girdled with the plant tops broken over but still partially connected to the root system.

Fifteen samples consisting of three plants each were taken from each group on September 23, 1977. The stage of development of the plants was R6. The plant tops were clipped from the roots and discarded. Each sample, comprised of three complete root systems, was carefully washed in a pail of tap water and placed in a jar for the $C_2H_2$ reduction assay. Nodule number and dry nodule weight per plant as well as nitrogenase activity were determined for each sample.

The following summer a field of 'Forrest' soybeans near Port Allen, La. was chosen for a second experiment to gain further evidence as to the detrimental effects of three-cornered alfalfa hopper damage on soybeans. The plants in this field had received heavy damage early in the season as a result of stem girdling caused by this insect.

In this experiment only two groups of plants were sampled:

Group a. Plants that were undamaged and

Group b. Plants that had been completely girdled but were not lodged.

On July 6, 1978, 34 samples of three plants each were taken from each group. The plants were in the R1 stage of development. The plant tops were clipped at the soil level and each sample of three root systems was placed in a jar for the $C_2H_2$ reduction assay. The total leaf area of each plant was determined with the area meter and the entire plant tops were dried for five days at 60$^o$C and later analyzed for total-N (Bremner, 1965). Nodule number, nodule dry weight and nitrogenase activity were determined for each sample.

Also on July 6, 1978, a survey of the plants in the field was conducted by Dr. L. D. Newsom of the Department of Entomology at LSU to determine the number of plants that had been damaged by the three-cornered alfalfa hopper. At 15 randomly selected locations within this field, all of the plants within a 75 cm segment of row were carefully inspected for damage caused by this insect. The total number of plants and the number of girdled plants were determined for each row segment.

On August 2, 1978, a second experiment was conducted in the same field to determine the effects of stem girdling on $N_2$ fixation during the R5 stage of development. In this study the plants were divided into three groups on the basis of damage caused by this insect. The groups were as follows:

Group a. Control plants that had not been visibly damaged.

Group b. Plants that had been girdled but were not lodged.

Group c. Plants that had been girdled with the plant tops broken over but still partially connected to the root system.

Twelve plants were randomly selected from each group and the tops were clipped from the roots and discarded. One complete root system was placed in each jar for the $C_2H_2$ reduction assay. Nodule number, nodule dry weight and the parameters for nitrogenase activity were determined for each sample.

A final experiment was conducted in a field of 'Bragg' soybeans on August 2, 1978, to study the effects of three-cornered alfalfa hopper damage on $N_2$ fixation by soybeans. As in the previous study, the plants in this field had received heavy damage due to stem girdling by this insect.

Plants included in this study were placed in one of the following three groups:

Group a. Plants which had received no apparent damage from the three-cornered alfalfa hopper.

Group b. Plants that had been girdled but were not broken over.

Group c. Plants that had been completely girdled by the three-cornered alfalfa hopper and the stems were broken over.

Twelve plants representing each group were carefully excavated from the soil on August 2, 1978. The stage of development of the plants was R2. The plant tops were clipped off and discarded. The root systems were carefully washed and one root system was placed in each jar for the $C_2H_2$ reduction assay. As in previous experiments, nodule number, nodule weight and nitrogenase activity were determined for each sample.

B. Nodule Damaging Insect Larvae Tests

A field study was conducted on the Dan Richard farm near Port Barre, La. in 1976 to study the effects of nodule damage caused by larvae of the bean leaf beetle, banded cucumber beetle and the platystomatid fly on $N_2$ fixation by soybeans. The soil type on which the study was conducted was a Sharkey clay. The field was planted to 'Forrest' soybeans on April 22, 1976.

Small screen cages approximately 1 x 1 x 1 m were placed over one month old soybean plants and the adults of these insects were introduced into the cages. All cages were placed on the same row with approximately 2 m between each cage. The treatments included in this study were set up by Dr. Catherine Eastman, formerly with the Department of Entomology at LSU and presently at the University of Illinois. Each insect treatment consisted of three experimental units (cages) in a completely randomized design. The treatments are as follows:

Treatment 1. Adults of the platystomatid fly were introduced in to each cage on nine dates between May 20, 1976 and July 3, 1976. A total of approximately 300 of these insects were put in each cage. Two 250 ml plastic bottles filled with 10% sucrose solution were fitted with sponge wicks and suspended from the side of each cage to offer a food supply for the adult flies.

Treatment 2. Approximately 64 adult bean leaf beetle adults were put in each cage on May 20, 1976 and again on May 25, 1976.

Treatment 3. On four dates from May 20, 1976 to June 9, 1976, a total of 192 adults of the banded cucumber beetle were put into each cage.

Treatment 4. The soil within the cages was liberally treated with carbofuran granules which were worked into the soil to kill existing soil insects. No insects were added to these cages.

Treatment 5. Uncaged control plants were taken from the vicinity of the cages.

Treatment 6. Uncaged control plants were taken approximately 50 m into the field from the cages.

On July 13, 1976, four plants were carefully excavated from each cage and the plant tops were clipped off and discarded. The stage of development of the plants was R2. Two complete root systems were washed and put into each jar to serve as a single sample for the $C_2H_2$ reduction assay. Dr. Eastman removed the nodules from the roots and examined each nodule under a dissecting microscope for insect feeding damage. As in previous experiments, nodule number, nodule dry weight and nitrogenase activity were determined as described previously.

Two investigations were conducted during 1977 to study the effects of nodule injury caused by natural populations of nodule damaging insect larvae on nodulation and $N_2$ fixation by soybeans. A field of 'Dare' soybeans on the Dan Richard farm near Krotz Springs, La. was chosen for the first study because it was known to have a history of consistently high populations of bean leaf beetles. This field was planted on April 25, 1977.

The test consisted of eight plots 10 m long with four rows spaced 100 cm apart. Four of the plots were treated with both carbofuran and aldicarb when the plants were in the unifoliate stage and again two weeks later. On both application dates 2.2 kg/ha (active ingredient) of each insecticide was applied to these plots. The insecticides were applied to the surface of the plots in a band extending about 10 cm on each side from the base of the plants. The remaining four plots were left untreated to serve as controls. Therefore, the experiment consisted of four completely randomized replications of treated and untreated plots. One application of acephate was applied to all plots in late August to control velvetbean caterpillars. Dr. L. D. Newsom of the Department of Entomology at LSU set up the field experiment and applied all insecticides.

Ten plants were taken from each plot on June 8, 1977, and the leaf area of each plant was determined with the area meter. The stage of development of the plants was V6. The plant tops were oven dried for three days at 60°C, and the total-N content was determined as described previously. At the time these samples were taken, high populations of the soybean thrips, Sericothrips variabilis (Beach) were noted in the control plots. Very few of these insects were found in the plots with carbofuran and aldicarb.

On June 15, 1977, four samples with three plants each were taken from each of the plots. The top of each plant was clipped from the roots and discarded. The entire nodulated root systems were washed in a pail of tap water and the $C_2H_2$ reduction assay was performed. Number and dry weight of nodules per plant was determined and the usual parameters for nitrogenase activity were obtained as described previously. Ten days later, on June 25, 1977, 10 plants were selected at random from each plot and the leaf area per plant was determined in the laboratory with an area meter.

On July 13, 1977 these plots were again sampled as on June 15 and the same parameters were measured. The stage of development of the plants was R2.

On September 15, 1977 the two center rows of each plot were harvested by hand and the plants were brought to the greenhouse and allowed to air-dry. On September 27, 1977, the plants were run through a "small-plot" thresher and the seed yield was determined to the nearest 0.05 kg. The average yield (kg/ha) at 13% moisture was then determined for both treatments.

A second experiment was conducted during 1977 to study the effects of nodule injury in soybeans caused by larvae of the bean leaf beetle. A field of 'Dare' soybeans on the Bill Pollingue farm near Port Barre, La. was chosen for this study. As in the previous experiment, this field had a history of high populations of bean leaf beetles. The field had been planted on May 2. The set up of the experiment, treatments and the dates of sampling were the same as in experiment 1. The usual parameters, nodule number, nodule dry weight and nitrogenase activity were determined.

A final investigation was conducted near St. Gabriel, La. in 1978 to determine the effects of bean leaf beetle larvae on nodulation and $N_2$ fixation by soybeans grown in screen cages. Five 16.7 x 16.7 x 2.4 m screen cages were used in this study. Five 12.2 m long rows in each cage were planted to 'Forrest' cultivar and another five 12.2 m long rows were planted to 'Bragg'. Both cultivars were planted on May 22, 1978. The soil in all cages was a Sharkey clay. On June 20, 1978, various numbers of bean leaf beetle adults were introduced into four of the cages. The remaining cage was used as an undamaged control. The numbers of adult bean leaf beetles added to the cages on June 20, 1978, were as follows:

Cage 1 - - - - 5340

Cage 2 - - - - 3200

Cage 3 - - - - 1100

Cage 4 - - - - 550

Cage 5 - - - - none

On July 10, 1978 the numbers of bean leaf beetles in each cage were supplemented with the following numbers of adults per cage:

Cage 1 - - - - 3000 making a total of 8340

Cage 2 - - - - 1000 making a total of 4200

Cage 3 - - - - 600 making a total of 1700

Cage 4 - - - - 500 making a total of 1050

Cage 5 - - - - none

On July 12, 1978 five samples composed of three plants each were randomly taken from each cage. In this study only the 'Forrest' cultivar was sampled. These plants were in the R2 stage of development. The plant tops were clipped off at the soil level and the entire root system of each plant was gently washed in water to remove most of the adhering soil particles. Each sample, containing three complete root systems was placed in a jar and the $C_2H_2$ reduction assay was performed. The total leaf area of the plant tops was determined with an area meter after which the leaves were dried for three days at $60^{o}C$, ground to pass through a 2 mm screen and analyzed for total-N. Nodule number per plant, nodule dry weight per plant and nitrogenase activity were determined as described previously.

Both 'Forrest' and 'Bragg' cultivars were sampled on August 17, 1978 to again determine the effects of bean leaf beetle damage on the same parameters measured on July 12, 1978. In this study, however, the total-N content of the soybean leaves was not determined. Ten samples of each cultivar were taken from each cage. Each sample contained a single plant. The 'Forrest' and 'Bragg' soybeans were in the R4 and R3 stages of development, respectively.

On September 12, 1978, both cultivars were again sampled with five samples per variety being taken from each cage. Again each sample was composed of a single plant. The stages of development of the 'Forrest' and 'Bragg' soybeans were R6 and R5, respectively. The usual parameters for nitrogenase activity, nodule number and nodule dry weight were determined for each sample.

III. RECOVERY OF *RHIZOBIUM JAPONICUM*, STRAIN 110, IN SOYBEAN NODULES.

A three year study was undertaken to determine the extent to which a non-indigenous strain of *Rhizobium japonicum*; strain 110, could be introduced into the nodules of soybeans when two different rates of this strain were used as an inoculant. Nodulating and non-nodulating isolines of 'Lee' soybeans were included in the study so that the contributions of N from the soil and from $N_2$ fixation could be estimated. Fertilizer N was applied to the nodulating and non-nodulating soybeans so that $N_2$ fixed by the nodulating soybeans could be expressed on a fertilizer N basis and to determine what, if any, effects fertilizer N would have on $N_2$ fixation, nodulation and yield of nodulating soybeans.

The study was begun in 1976, at the Burden Research Center in Baton Rouge, La. The soil in this location was an Olivier silt loam which contained approximately $10^5$ *R. japonicum*/g of soil (S. Scott, unpublished data). Some properties of this soil are listed in Table 2. A total of 42 field plots were utilized in this study which consisted of six replications of seven treatments in a randomized block design. Each field plot consisted of four rows, 6.1 m long spaced 1.0 m apart. All plots were planted on June 10, 1976. The treatments included in this study are as follows:

Treatment 1. Nodulating 'Lee' soybeans (1 seed/2.5 cm) were planted without inoculation with *R. japonicum*, and no fertilizer-N was applied to these plots (Control).

Treatment 2. Nodulating 'Lee' soybeans were dropped into an open furrow (1 seed/2.5 cm) and were inoculated with $10^8$ viable *R. japonicum*, strain 110, cells per cm of furrow. The liquid inoculant was dribbled into the open furrow by a gravity flow applicator. The seeds were then covered with approximately 2 cm of soil. No fertilizer-N was applied (N-$10^8$).

Treatment 3. Same as Treatment 2 except only $10^4$ cells/cm of row were applied (N-$10^4$).

Treatment 4. Nodulating 'Lee' soybeans were inoculated with Nitragin® peat-base inoculant and planted at the rate of 1 seed/2.5 cm Nitragin®. No fertilizer-N was applied (N-P).

Treatment 5. Same as Treatment 4 except the equivalent of 250 kg/ha of $NH_4NO_3$-N was broadcast on the soils' surface of each plot. The N was applied in five equal increments on June 10, 1976, (at planting), July 23, 1976, August 10, 1976, August 17, 1976 and August 30, 1976 (N-P-250).

Treatment 6. Non-nodulating 'Lee' soybeans were planted without seed inoculation and no fertilizer-N was applied (NN-0).

Treatment 7. Non-nodulating 'Lee' soybeans were planted without seed inoculation, and 250 kg/ha of $NH_4NO_3$-N was applied during the growing season in five equal increments on the same dates as given previously (NN-250).

Table 2. Some properties of the Olivier silt loam soil used for field studies on recovery of *R. japonicum*, strain 110, in root nodules.

| Olivier Silt Loam | |
|---|---|
| Soil reaction (pH) | 5.8 |
| Organic matter | 0.85% |
| Extractable P | 53 ppm |
| Extractable K | 87 ppm |
| Extractable Ca | 870 ppm |
| Extractable Mg | 348 ppm |

The *R. japonicum*, strain 110, inoculant was obtained from Ag. Labs of Columbus, Ohio in 1976 and 1977 and from Microlife Technics of Sarasota, Florida in 1978. This inoculant contained $10^{13}$ viable *R. japonicum* cells/ml. It was kept frozen at -15°C until it was needed in the field. The frozen concentrate was diluted to the desired concentration with a special buffer solution containing 0.27 g/l of $KH_2PO_4$, 0.34 g/l of $K_2HPO_4$ and 5 g/l of sucrose.

On August 12, 1976, the $C_2H_2$ reduction assay was conducted on three plants randomly selected from the outer two rows of each plot. The stage of development of the plants was R2. Nodule number and dry weight were determined for the plants in each sample and the parameters for nitrogenase activity were determined. Plant height measurements were taken in each plot on August 13, 1976, by measuring the height of 10 randomly selected plants in each plot.

On August 16, 1976, three nodulated root systems were collected from the outer two rows of the control, N-$10^4$ and N-$10^8$ plots. The root systems were washed in water, sealed in sterile polyethylene bags and stored in a deep freeze at -15°C. On April 4, 5 and 6, 1978, a random sample of 24 nodules from each plot was analyzed serologically to determine the recovery of serogroup 110 in the root nodules of each treatment.

A modified form of the procedure developed by Means et al. (1964) was used to determine the recovery of applied R. japonicum strain 110 in the soybean root nodules. The procedure used in this study is summarized briefly below.

The root nodules were removed from the root system and washed under a stream of tap water to remove adhering soil particles. The washed root nodules were then placed in 10 ml glass tubes containing 2 ml of 0.55% NaCl solution (saline). Each nodule was thoroughly crushed, and the resulting cell suspensions were steamed for 30 minutes at 90°C to destroy the flagellar (H) antigen. Following this, the cell suspensions were allowed to cool and were adjusted to an optical density of 0.6 with 0.55% saline. Optical density was measured with a Bausch and Lomb Spectronic 20 Spectrophotometer set at 520 nm.

A pipette was used to dispense 0.025 ml of 0.55% saline and 0.025 ml of R. japonicum, strain 110, antiserum into each well of a microtiter plate. The antiserum was diluted with 0.55% saline. In 1976 and 1977 the ratio of saline to antiserum used was 50:1. In 1978 a ratio of 24:1 was used. Then 0.025 ml of cell suspension was added to each well of the microtiter plate. A separate well was used for each nodule cell suspension. The microtiter plates were then sealed with cellophane tape and mixed using a gentle swirling motion.

The plates were incubated in a water bath for 18 hours at 53°C after which each well was observed under a dissecting microscope. Each well was rated either positive or negative for the presence of agglutination of the cell suspension. A positive rating for a cell suspension indicated the presence of serogroup 110.

The two inner rows of each plot were harvested on November 16, 1976, and the seed yield (kg/ha), corrected to 13% moisture, was determined for each plot. A random sample of the seeds from each plot was dried for five days at 60°C, and the total-N content of the seeds was determined. Percent protein in the seeds was estimated by multiplying percent N by 6.25.

The average seed yield per treatment was multiplied by the percent N in the seeds to determine the amount of seed N in each treatment. The contribution of seed N attributed to $N_2$ fixation was obtained by subtracting the seed N (kg/ha) in the NN-0 treatment from the seed N (kg/ha) in the control, N-$10^4$, N-$10^8$ and N-P treatments.

In 1977 the same treatments were used as in 1976 and each treatment was located on the same plots. The dates of fertilizer application were June 20, July 7, July 22, August 1, and August 12, 1977. The $C_2H_2$ reduction assay was performed on July 18, 1977 when the plants were in the R2 stage of development. The procedure was carried out exactly as in 1976. In this study, however, the plant tops were saved, dried for three days at 60°C and analyzed for total-N.

Nodules were collected for serotyping on August 1, 1977 and the serological tests were conducted on July 6, 28 and 29, 1978. The same procedure was used as for the nodules collected in 1976.

The two middle rows of each plot were harvested on November 11, 1977, and the seed yield, corrected to 13% moisture was determined for each plot. The total-N content of the seeds was determined and the percent protein was estimated. The contribution of seed N attributed to $N_2$ fixation was also estimated.

In 1978, the third year of the study, the same treatments were used as in 1977; however, one additional treatment was added. This treatment, also having six replications, was the same as N-$10^8$ except that it was fertilized with the equivalent of 250 kg/ha of $NH_4NO_3$-N. This treatment was designated N-$10^8$-250. All of the treatments were planted on May 25 and 26, 1978 in exactly the same plots as in 1976 and 1977. The new treatment was planted in adjacent plots which had not been used previously.

The dates of fertilizer application were July 7, July 20, August 4, August 18, and September 1, 1978. The $C_2H_2$ reduction assay was performed on July 26, 1978 when the plants were in the R2 stage of development. On August 1, 1978 the root samples were collected from the control, N-$10^4$, N-$10^8$, and N-$10^8$-250 treatments for serological identification of the nodule bacteria. The serological tests were conducted on September 18, 19 and 20, 1978.

The two middle rows of each plot were harvested on October 25, 1978 and the seed yield, corrected to 13% moisture, was determined for each test plot. Seed N and percent protein were determined for each treatment as in 1976 and 1977. The contribution of N from $N_2$ fixation was also calculated for the control, N-$10^4$, N-$10^8$ and N-P treatments.

## RESULTS AND DISCUSSION

### I. GREENHOUSE STUDIES ON THE EFFECTS OF INSECT DAMAGE ON NODULATION AND $N_2$ FIXATION BY SOYBEANS

Section I of the Results and Discussion will include the results of greenhouse experiments conducted in order to study the effects of various types of insect damage on nodulation and $N_2$ fixation ($C_2H_4$ produced) by soybeans. The results of each study will be presented separately, and a discussion will appear following the results of each type of insect damage.

Types of insects used in the greenhouse investigations included the soybean looper (*Pseudoplusia includens*), three-cornered alfalfa hopper (*Spissistilus festinus*) and the southern green stink bug (*Nezara viridula*).

#### A. Soybean Looper Defoliation Tests

Two experiments were conducted in the greenhouse to study the effects of soybean looper defoliation on nodulation and $N_2$ fixation by soybeans. In the first experiment, four soybean loopers were placed on each leaf of the looper-damaged plants and allowed to feed for 14 days. The experiment consisted of 19 replications of looper-damaged and control plants. Each experimental unit was composed of three plants which were in the V5 stage of development.

Partial defoliation of the soybean plants caused by the soybean looper resulted in a significant decrease in leaf area per plant (Table 3). The decrease in leaf area on the looper-damaged plants

Table 3. Effects of soybean looper defoliation on leaf area, nodulation and $C_2H_4$ production by greenhouse-grown soybeans; means for 19 replications.

| Treatment | Leaf area per plant | Nodule dry weight per plant | Nodules per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule | $C_2H_4$ per $cm^2$ of leaf area |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | -mg- | -number- | - - - - - - - - - - - - - - $\mu M$ - - - - - - - - - - - - - - | | | |
| Control | 299.1 | 155.0 | 35.6 | 8.73 | 0.25 | 56.4 | 0.029 |
| Looper damaged | 183.8** | 133.3** | 36.3 | 5.67** | 0.16** | 43.0** | 0.031 |

** Smaller than the control at the 1% level of probability.

was approximately 38% as compared to the undamaged control plants. Nodule number per plant was not significantly affected by the looper damage; however, the average dry weight of nodules per plant was significantly reduced. A significant reduction in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule was noted in the looper-damaged plants. These parameters were reduced by 35, 24 and 36%, respectively. No significant differences in $N_2$ fixed ($C_2H_4$ produced) per $cm^2$ of leaf area were detected between the control and looper-damaged plants.

A second experiment was conducted in the greenhouse to study again the effects of partial insect defoliation on nodulation and $N_2$ fixation by soybeans. In this study four soybean loopers were placed on half of the plants and allowed to feed for 10 days, after which the $C_2H_2$ reduction assay was performed. The stage of development of the plants was V5. There were 10 replications of looper-damaged and control plants. Each experimental unit consisted of three plants.

As in the previous study, leaf area per plant was significantly decreased by the soybean loopers (Table 4). Significant reductions in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per $cm^2$ of leaf area were also noted in the looper-damaged plants. These values were reduced by 37, 68 and 64%, respectively, as compared to the control. No significant decrease in $N_2$ fixed ($C_2H_4$ produced) per g of dry nodule was detected, although this parameter was decreased by 55%. Average nodule number and nodule dry weight per plant was not significantly different for the treatments.

Table 4. Effects of soybean looper defoliation on leaf area, nodulation and $C_2H_4$ production by greenhouse-grown soybeans; means for 10 replications.

| Treatment | Leaf area per plant | Nodule dry weight per plant | Nodules per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule | $C_2H_4$ per $cm^2$ of leaf area |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | -mg- | -number- | - - - - - - - - | - - - - - - - $\mu M$ | - - - - - - - - | - - - - - - |
| Control | 202.5 | 63.7 | 20.7 | 2.42 | 0.124 | 36.4 | 0.012 |
| Looper damaged | 128.5** | 50.4 | 17.9 | 0.78** | 0.044** | 16.5 | 0.006** |

** Smaller than the control at the 1% level of probability.

Interpretation of the results of these two studies indicated that partial defoliation of soybeans caused by the soybean looper substantially decreased $N_2$ fixation ($C_2H_4$ produced) by soybean plants. This decrease in $N_2$ fixation was attributed to the reduction in photosynthate produced by damaged plants due to the destruction of significant amounts of leaf tissue.

B. Simulated Insect Defoliation Versus Soybean Looper Defoliation Tests.

Five experiments were conducted in the greenhouse to study the effects of two types of simulated insect defoliation of soybeans and to determine if the methods used were valid means of simulating insect defoliation damage.

The objective of the first experiment was to compare two types of mechanical defoliation of soybeans and to study the effects of these types of defoliation on nodulation and $N_2$ fixation ($C_2H_4$ produced) by soybeans.

This experiment consisted of plants that had been mechanically injured by removing one leaflet from each trifoliate leaf (Clipped), plants that had been mechanically damaged by punching holes in each leaflet (Punched) and plants that were left undamaged to serve as controls. There were 10 experimental units in each treatment with three plants each. The plants were damaged six days prior to harvest when the stage of development of the plants was V6.

In this study the two types of simulated insect defoliation (Clipped and Punched) caused significant reductions in leaf area per plant compared to the control (Table 5). No significant differences

Table 5. Effects of two types of simulated insect damage on leaf area, nodulation and $C_2H_4$ production by greenhouse-grown soybeans; means for 10 replications.

| Treatment | Leaf area per plant | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule | $C_2H_4$ per $cm^2$ of leaf area |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | -number- | -mg- | - - - - - - - - - - - - | - - - - - - - - µM | - - - - - - - - | - - - - - - - - - - - - |
| Control | 542.4 | 49.7 | 195.1 | 6.3 | 0.146 | 33.3 | 0.0115 |
| Punched[1/] | 388.6 | 42.9 | 168.4 | 6.4 | 0.134 | 33.8 | 0.0170 |
| Clipped[2/] | 334.8 | 45.5 | 192.4 | 6.9 | 0.161 | 40.7 | 0.0208 |
| L.S.D. (0.05) | 65.7 | | | | | | 0.0038 |
| L.S.D. (0.01) | 88.7 | | | | | | 0.0051 |

1/ Holes were punched in each leaflet with a paper punch.

2/ One-third of the leaflets were removed from each plant.

in leaf area per plant existed between the Punched and Clipped treatments; however, the amount of $N_2$ fixed ($C_2H_4$ produced) per $cm^2$ of leaf area was significantly higher in the Clipped treatment than in the Punched treatments. It was noted that, with regard to this parameter, the control was significantly lower than both of the damaged treatments. This indicated that some factor other than leaf area per plant may have been limiting $N_2$ fixation in this experiment. The data indicated that nodule number was also not responsible for the differences. Nodule weight was slightly less for the Punched treatments but was very close when the control was compared to the Clipped dry weight. No significant differences were detected in number of nodules per plant, weight of nodules per plant or $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule or per g of dry nodule weight.

A second study was performed in the greenhouse to compare again the previously mentioned methods of simulated insect damage and to compare the results to actual soybean looper damage. In this study the plants were harvested eight days after they were damaged. At harvest, the stage of development of the plants was V6. Each treatment consisted of seven experimental units with two plants each. As shown in Table 6, leaf area per plant in each of the damaged treatments was significantly lower than the control. No significant differences in leaf area per plant existed between the two types of simulated insect damage. The looper-damaged plants, however, were significantly lower in leaf area per plant than the other treatments. None of the treatments produced any significant differences in nodule number or nodule dry weight per plant. The looper-damaged plants were

Table 6. Effects of two types of simulated insect defoliation and soybean looper defoliation on nodulation, leaf area and $C_2H_4$ production by soybeans grown in the greenhouse; means for seven replications.

| Treatment | Leaf area per plant | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule | $C_2H_4$ per $cm^2$ of leaf area |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | -number- | -mg- | uM | | | |
| Control | 529.2 | 54.9 | 243 | 14.7 | 0.281 | 60.0 | 0.0278 |
| Punched | 395.3 | 44.8 | 212 | 11.1 | 0.251 | 54.1 | 0.0279 |
| Clipped | 422.9 | 55.7 | 223 | 12.7 | 0.234 | 57.7 | 0.0300 |
| Looper damaged | 207.2 | 45.3 | 162 | 3.3 | 0.074 | 19.6 | 0.0157 |
| L.S.D. (0.05) | 32.33 | | | 2.53 | 0.074 | 11.52 | 0.0060 |
| L.S.D. (0.01) | 43.80 | | | 3.43 | 0.101 | 15.62 | 0.0081 |

significantly lower than the control plants in all parameters of nitrogenase activity. Punching holes in each leaflet of the soybean plants (Punched) significantly reduced the production of $C_2H_4$ per plant, but none of the other parameters of nitrogenase activity were significantly different from the control. The only parameter that was significantly reduced by removing one leaflet from each trifoliate leaf (Clipped) was leaf area per plant.

No significant differences for any parameter were noted between the two types of simulated insect damage. Due to the great difference in the amount of defoliation between the looper-damaged plants and the mechanically-damaged plants, it was concluded that no meaningful comparison could be made between these treatments. Based on these data, therefore, no conclusions were made as to whether or not the two methods of mechanical defoliation were valid means of simulating damage caused by insects such as the soybean looper.

A third experiment was conducted in the greenhouse to study the effects of simulated insect defoliation and soybean looper defoliation on nodulation and $N_2$ fixation by soybeans. This experiment included the same treatments as the preceding experiment. In this study, however, the plants were harvested only three days after they had been partially defoliated. The treatments were replicated 10 times, and each experimental unit consisted of two plants in the V6 stage of development.

The data indicated that the plants from both of the mechanically-damaged treatments received approximately equal amounts of defoliation damage (Table 7). The plants injured by the soybean loopers, however, were damaged to a much greater extent than either of the mechanically-

Table 7. Effects of two types of mechanical defoliation and soybean looper defoliation on nodulation and $C_2H_4$ production by greenhouse-grown soybeans; means for 10 replications.

| Treatment | Leaf area per plant | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule | $C_2H_4$ per $cm^2$ of leaf area |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | -number- | -mg- | | | -µM- | |
| Control | 403.4 | 54.1 | 174.5 | 9.35 | 0.179 | 53.3 | 0.023 |
| Punched | 308.0 | 48.5 | 159.5 | 6.30 | 0.132 | 39.4 | 0.020 |
| Clipped | 289.1 | 50.7 | 168.0 | 6.75 | 0.137 | 40.6 | 0.023 |
| Looper damaged | 239.9 | 49.7 | 164.0 | 4.45 | 0.092 | 27.9 | 0.019 |
| L.S.D. (0.05) | 40.63 | | | 1.13 | 0.028 | 5.57 | 0.0037 |
| L.S.D. (0.01) | 45.45 | | | 1.52 | 0.038 | 7.48 | 0.0050 |

damaged plants. There were no differences among the treatments in either nodule number or nodule dry weight per plant. However, all values of nitrogenase activity were significantly lower than the control with the exception of $C_2H_4$ produced per $cm^2$ of remaining leaf surface area.

A fourth experiment was conducted to study again the effects of soybean looper defoliation and simulated insect defoliation of soybeans. In this study only one type of simulated insect damage (Clipped) was included. It was hoped that in this study approximately equal amounts of defoliation could be achieved on the looper-damaged and mechanically-damaged plants so that the effects of these types of defoliation on nodulation and $N_2$ fixation could be compared. This experiment differed from all previous experiments in that the loopers were allowed to partially defoliate the plants for four days after which they were removed from each plant by hand. On the same day that the loopers were removed from these plants, the mechanical damage was inflicted on the Clipped treatment. The experiment consisted of 10 replications of three treatments. Each experimental unit consisted of one plant. The root systems were harvested for the $C_2H_2$ reduction assay when the plants were in the V6 stage of development.

No significant differences were noted among the values for nodule number per plant or nodule dry weight per plant (Table 8). The looper-damaged plants and the Clipped plants received significant reductions in leaf surface area per plant as compared to the control. As a result of this damage, all parameters of nitrogenase activity were reduced significantly for these treatments. No significant differences were found

Table 8. Effects of mechanical defoliation and soybean looper defoliation on leaf area and $C_2H_4$ production by soybeans grown in the greenhouse; means for 10 replications.

| Treatment | Leaf area per plant | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|
| | -$cm^2$- | -number- | -mg- | | $\mu M$ | |
| Control | 212.0 | 24.8 | 102 | 2.89 | 0.129 | 29.0 |
| Looper damaged | 128.9 | 20.3 | 76 | 1.28 | 0.066 | 20.0 |
| Clipped | 166.1 | 26.5 | 118 | 1.54 | 0.065 | 14.2 |
| L.S.D. (0.05) | 46.4 | | | 0.77 | 0.049 | 8.32 |
| L.S.D. (0.01) | 62.6 | | | 1.04 | 0.066 | 11.24 |

to exist between the looper-damaged plants and the Clipped plants for any of the parameters included in this study.

A final experiment was conducted in the greenhouse to study the effects of soybean looper defoliation, simulated insect damage and time of defoliation on nodulation and $N_2$ fixation by soybeans. In this experiment leaves were mechanically defoliated or inoculated with soybean loopers five, three or one day before the roots were excavated for the $C_2H_2$ reduction assay.

As shown in Table 9, one day was sufficient time for the soybean loopers to significantly reduce the average leaf area per plant, $N_2$ fixed ($C_2H_4$ produced) per plant and per g of dry nodule. After three days $N_2$ fixed ($C_2H_4$ produced) per nodule was significantly reduced by the insects. Punching holes in the leaves with a hole puncher caused significant reductions in these same parameters as early as one day after the plants were damaged. When one-third of the leaflets were removed from each plant (Clipped 1/3) highly significant reductions in $N_2$ fixed ($C_2H_4$ produced) per plant and per g of dry nodule were detected after only one day. When two-thirds of the leaflets were removed (Clipped 2/3), even greater decreases in these parameters were noted.

Interpretation of the data in these five greenhouse experiments gave conclusive evidence that defoliation of soybeans by all methods tested caused substantial reduction in $N_2$ fixation ($C_2H_2$ reduction) as soon as one day after the damage occurred. None of the experimental data proved conclusively that "punching" or "clipping" of leaves are

Table 9. Effects of soybean looper defoliation, mechanical defoliation and time on number and dry weight of nodules and $C_2H_4$ production by soybeans grown in the greenhouse; means for six replications.

| Treatment | Leaf area per plant | Defoliation | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of of dry nodule | $C_2H_4$ per $cm^2$ of leaf area |
|---|---|---|---|---|---|---|---|---|
| | -$cm^2$- | - % - | -number- | -mg- | - - - - - - | - - - - - - uM | - - - - - - | - - - - - - |
| Loopers - 5 days | 199.9 | 31.5 | 29.5 | 71 | 4.88 | 0.17 | 72.4 | 0.025 |
| Loopers - 3 days | 166.2 | 43.0 | 26.0 | 72 | 4.36 | 0.17 | 60.9 | 0.026 |
| Loopers - 1 day | 227.8 | 21.9 | 26.9 | 81 | 5.70 | 0.22 | 69.9 | 0.025 |
| Punched - 5 days | 184.3 | 36.8 | 27.4 | 55 | 3.41 | 0.13 | 63.4 | 0.019 |
| Punched - 3 days | 200.3 | 31.3 | 35.1 | 74 | 3.69 | 0.12 | 49.7 | 0.018 |
| Punched - 1 day | 208.2 | 28.6 | 33.3 | 76 | 4.25 | 0.13 | 56.8 | 0.020 |
| Clipped 1/3-1 day | 162.2 | 44.4 | 25.9 | 68 | 3.37 | 0.17 | 53.5 | 0.026 |
| Clipped 2/3-1 day | 86.1 | 70.5 | 23.7 | 65 | 3.28 | 0.15 | 51.9 | 0.038 |
| Control | 291.4 | | 30.6 | 82 | 7.00 | 0.24 | 85.4 | 0.024 |
| L.S.D. (0.05) | 28.46 | | | 13.0 | 1.25 | 0.056 | 13.52 | 0.0048 |
| L.S.D. (0.01) | 38.08 | | | 17.4 | 1.67 | 0.074 | 18.02 | 0.0065 |

valid methods of simulating insect defoliation of soybeans. It should be understood, however, that mechanical damage to soybeans is unnatural in that maximum defoliation occurs within a matter of seconds or minutes, whereas damage caused by insects such as the soybean looper gradually increases during the test period. Also, the mechanically-damaged plants quickly began to compensate for the loss of leaf area by producing new leaves and by growth of the undamaged leaf portions of immature leaves; therefore, mechanical leaf damage in many instances may not be a valid method of simulating defoliation of soybeans caused by such insects as the soybean looper.

C. Three-Cornered Alfalfa Hopper Tests

Two greenhouse experiments were conducted to study the effects of stem girdling caused by the three-cornered alfalfa hopper on $N_2$ fixation ($C_2H_4$ produced) and nodulation of soybeans.

In the first experiment the test plants were subjected to continuous feeding by 10 adult three-cornered alfalfa hoppers for a period of 21 days. The stage of development of the plants when the root systems were harvested was V5. The experiment included 10 damaged plants and 10 undamaged plants (Controls).

Stem girdling of the soybean plants by this insect caused a significant reduction in dry nodule weight per plant and $N_2$ fixed ($C_2H_4$ produced) per plant (Table 10). No significant differences were noted for the other parameters.

The second experiment was basically the same, but the number of experimental units per treatment was increased to 15. The damaged plants were subjected to continuous feeding for a period

Table 10. Effects of stem girdling by the three-cornered alfalfa hopper on growth, nodulation and $C_2H_4$ production by 'Bossier' soybeans grown in the greenhouse; means for 10 replications.

| Treatment | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of of dry nodule | Dry weight of plant tops per plant |
|---|---|---|---|---|---|---|
| | -number- | -mg- | - - - - - - - - - - - $\mu$M - - - - - - - - - - - | | | -mg- |
| Control | 5.2 | 14.0 | 0.807 | 0.134 | 14.5 | 900 |
| Girdled | 1.8 | 2.0* | 0.084* | 0.077 | 25.0 | 750 |

* Smaller than the control at the 5% level of probability.

of 22 days. The stage of development of the plants when harvested for the $C_2H_2$ reduction assay was V6.

As shown in Table 11, nodule number and dry weight of nodules of the damaged plants was significantly lower than the control. These two parameters were reduced by 33 and 36% respectively. As in the previous experiment, $N_2$ fixed ($C_2H_4$ produced) per plant was significantly reduced by the stem damage. This value was decreased by 58%. Values for $N_2$ fixed ($C_2H_4$ produced) per nodule and per g of dry nodule were not significantly decreased in the damaged plants.

As a result of these studies, it was concluded that $N_2$ fixation ($C_2H_4$ produced) by soybeans may be significantly reduced when the stems are girdled by three-cornered alfalfa hoppers. This decrease in $N_2$ fixation ($C_2H_4$ produced) was attributed to a decrease in the amount of photosynthate translocated from the leaves to the root nodules. At the time of harvest the stems of all plants girdled by this insect were larger in diameter above the injured area than below it. This difference in stem diameter was believed to be a result of more rapid stem growth above the girdled area than below because of the difference in supply of photosynthate to those areas.

D. <u>Stink Bug Test</u>

A final greenhouse experiment was conducted to study the effects of stem damage caused by the southern green stink bug on $N_2$ fixation ($C_2H_4$ produced) by soybeans. The experiment consisted of 30 replications of damaged and control plants. The damaged plants were divided into two levels with 15 plants each. The plants in Level 1 were subjected to feeding damage caused by five stink bugs feeding on

Table 11. Effects of stem girdling by the three-cornered alfalfa hopper on growth, nodulation and $C_2H_4$ production by 'Bossier' soybeans grown in a greenhouse; means for 15 replications.

| Treatment | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule weight | Fresh weight of plant tops per plant |
|---|---|---|---|---|---|---|
| | -number- | -mg- | - - - - - - - - - - - | - - - - $\mu$M - - - - | - - - - - - - - - - - | - - - g - - - |
| Control | 8.4 | 33 | 0.977 | 0.115 | 26.2 | 4.15 |
| Girdled | 5.7* | 21* | 0.410* | 0.064 | 17.1 | 3.59 |

* Smaller than the control at the 5% level of probability

the stem of each plant for a period of nine days. The plants in Level 2 were subjected to feeding damage caused by five stink bugs for a period of seven days after which the number of stink bugs was increased to ten per plant for the remaining two days. In Level 1 and Level 2, the injury inflicted upon the plants was limited to plant stems below the first trifoliate leaf. All plants were harvested for the $C_2H_2$ reduction assay in the V4 stage of development.

Both levels of stink bug damage resulted in significant decreases in nodule dry weight per plant (Table 12). Similar reductions in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight were also noted. No significant differences were detected in nodule number per plant.

These data indicated that stink bugs are capable of removing sufficient amounts of photosynthate from the plant stems to cause substantial reduction in $N_2$ fixation ($C_2H_4$ produced). The decrease in nodule weight per plant indicated that nodule growth may be adversely affected also. No significant differences were found to exist between the two levels of damage.

## II. FIELD STUDIES ON THE EFFECTS OF INSECT DAMAGE ON $N_2$ FIXATION BY SOYBEANS

A number of experiments were conducted at various locations during 1976, 1977 and 1978 to study the effects of damage from several types of insects on nodulation, $N_2$ fixation, growth and yield of soybeans under natural conditions. Types of insect-induced damage that were investigated included stem girdling caused by the three-cornered alfalfa

Table 12. Effects of stink bug damage to stems of soybeans on nodulation and $C_2H_4$ production by soybeans grown in the greenhouse.

| Treatment | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|
| | -number- | -mg- | - - - - - - - - - - | µM - - - - - - - - - - | - - - - - - - - - - |
| Control | 11.2 | 23 | 1.699 | 0.140 | 65.33 |
| Damaged[1/] (Level 1) | 8.1 | 8 | 0.294 | 0.042 | 26.29 |
| Damaged[2/] (Level 2) | 9.1 | 12 | 0.318 | 0.033 | 18.02 |
| L.S.D. (0.05) | | 7.30 | 0.685 | 0.056 | 18.37 |
| L.S.D. (0.01) | | 9.70 | 0.911 | 0.075 | 24.44 |

1/ Five stink bugs were placed on each plant stem.

2/ Ten stink bugs were placed on each plant stem.

hopper (*Spissistilus festinus*) and nodule damage caused by larvae of the bean leaf beetle (*Cerotoma trifurcata*), banded cucumber beetle (*Diabrotica balteata*) or the platystomatid fly (*Rivellia quadrifasciata*). Data relative to the damage caused by each of these insects are placed in a separate section entitled with a heading indicative of the type insect damage included therein.

A. Three-Cornered Alfalfa Hopper Tests

Four field experiments were conducted to study the effects of stem girdling by the three-cornered alfalfa hopper on nodulation and $N_2$ fixation ($C_2H_4$ production) by soybeans. In each of these experiments a number of plants had been visibly damaged (girdled) by the three-cornered alfalfa hopper and an equal number of plants which showed no visible signs of injury from this insect, were analyzed for nitrogenase activity. In all except one of the studies, damage to the soybean plants resulting from stem girdling was divided into two levels. Plants that had been completely girdled but were not lodged comprised the first level, and the second level consisted of girdled plants that had lodged but with the tops remaining partially connected to the root system.

The first experiment was conducted in a field of 'Bragg' soybeans near Port Barre, La., on September 23, 1977. Fifteen samples each of undamaged control plants, girdled plants that had not lodged and girdled plants that had lodged were carefully excavated from the soil and used in the $C_2H_2$ reduction assay. Each sample consisted of three plants that were in the R6 stage of development. Results of this experiment are shown in Table 13. Interpretation of the data indicated that both levels

Table 13. Effects of stem girdling by the three-cornered alfalfa hopper on nodulation and $C_2H_4$ production by field-grown 'Bragg' soybeans; means for 15 replications.

| Treatment | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|
| | -number- | -mg- | | - - - - - - μM - - - - - - | |
| Control | 114.62 | 526.33 | 10.97 | 0.097 | 21.7 |
| Girdled, not lodged | 70.62 | 292.67 | 5.87 | 0.086 | 20.9 |
| Girdled and lodged | 48.29 | 161.00 | 2.43 | 0.054 | 16.9 |
| L.S.D. (0.05) | 20.93 | 84.5 | 1.87 | 0.020 | |
| L.S.D. (0.01) | 28.00 | 113.0 | 2.50 | 0.027 | |

of three-cornered alfalfa hopper damage significantly reduced amounts of $N_2$ fixed ($C_2H_4$ produced) per plant, nodule number per plant and nodule dry weight per plant. The girdled plants that had been lodged were significantly lower than the control plants and the unlodged girdled plants with respect to $N_2$ fixed ($C_2H_4$ produced) per nodule. The values for the unlodged, girdled plants were not significantly different from the control. The average values for $N_2$ fixed ($C_2H_4$ produced) per g of dry nodule by the lodged and unlodged girdled plants were not significantly different from the control value.

A second test was conducted July 6, 1978, in a field of 'Forrest' soybeans near Port Allen, La. The field had received moderate to heavy girdling damage by the three-cornered alfalfa hopper. Stage of development of the plants was R1. In this study, 34 samples each of undamaged control plants and girdled plants that had not been lodged were taken from the field, with each sample consisting of three plants. No lodged plants were included in this study. As revealed in Table 14, the girdling damage caused by the three-cornered alfalfa hopper brought about a significant reduction in the average leaf area per plant. Although no significant differences were noted for any of the other parameters, all values for the girdled plants were lower than the control values. Nodulation and $N_2$-fixation ($C_2H_4$ production) values were very low in the girdled plants and the control plants.

A survey on July 6, 1978, by Dr. L. D. Newsom of the Department of Entomology at LSU revealed that 45% of the soybean plants in this

Table 14. Effects of stem girdling by the three-cornered alfalfa hopper on leaf area, N content, nodulation and $C_2H_4$ production by field-grown 'Forrest' soybeans; means for 34 replications.

| Treatment | Leaf area per plant | N content of leaves | Nodules per plant | Nodule wt. per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of nodule wt. |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | -%- | -number- | -mg- | | $\mu$M | |
| Control | 4034.67 | 5.40 | 16.8 | 41.67 | 0.90 | 0.054 | 22.2 |
| Girdled, not lodged | 2587.65** | 5.28 | 14.5 | 33.0 | 0.75 | 0.046 | 20.9 |

** Different from the control at the 1% level of probability.

field had been girdled by the three-cornered alfalfa hopper.

The results of a third study which was conducted in the same field on August 2, 1978, are presented in Table 15. The experiment consisted of 12 samples each of undamaged control plants, girdled plants that were not lodged and girdled plants that had lodged as a result of the insect damage. Each sample consisted of a single plant in the R 5 stage of development. No significant differences were detected between the treatments in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule or per g of dry nodule. The plants which had been girdled and were lodged had a significantly lower nodule number per plant and dry nodule weight per plant than the control plants.

A fourth experiment was conducted in a field of 'Bragg' soybeans near Port Allen, La., on August 2, 1978. The experiment consisted of 12 samples each of undamaged control plants, plants that had been girdled but were not lodged and plants that had been girdled and had lodged as a result of insect damage by the three-cornered alfalfa hopper. Each sample consisted of a single plant in the R2 stage of development. The data relative to this experiment are summarized in Table 16. No significant differences were detected between the control plants and those that had been girdled but had not lodged. The girdled plants that had lodged, however, were found to have values significantly lower than the control plants and the unlodged girdled plants in every parameter.

Although the results of these four field experiments were not consistent, they tended to confirm conclusions derived from the greenhouse studies that stem girdling caused by the three-cornered alfalfa

Table 15. Effects of stem girdling by the three-cornered alfalfa hopper on nodulation and $C_2H_4$ production by field-grown 'Forrest' soybeans; means for 12 replications.

| Treatment | Nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|
| | -number- | -mg- | | $\mu$M | |
| Control | 78.3 | 341 | 7.92 | 9.46 | 22.3 |
| Girdled, not lodged | 58.8 | 230 | 4.78 | 7.88 | 20.9 |
| Girdled and lodged | 41.8 | 131 | 3.56 | 7.75 | 25.3 |
| L.S.D. (0.05) | 23.9 | 126 | | | |
| L.S.D. (0.01) | 32.2 | 167 | | | |

Table 16. Effects of stem girdling by the three-cornered alfalfa hopper on nodulation and $C_2H_4$ production by field-grown 'Bragg' soybeans; means for twelve replications.

| Treatment | Nodules per plant | Nodule wt. per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|
| | -number- | -mg- | | μM | |
| Control | 75.9 | 454 | 10.50 | 13.11 | 22.54 |
| Girdled, not lodged | 74.5 | 446 | 9.48 | 13.35 | 20.66 |
| Girdled and lodged | 44.5 | 237 | 2.50 | 5.55 | 12.77 |
| L.S.D. (0.05) | 20.8 | 156 | 5.28 | 4.26 | 6.19 |
| L.S.D. (0.01) | 28.0 | 210 | 7.10 | 5.74 | 8.34 |

hopper reduced nodulation and fixation of $N_2$ ($C_2H_4$ production) by soybeans. Damage to nodulation and $N_2$ fixation ($C_2H_2$ reduction) was much greater in girdled plants that had lodged than in plants that had been girdled but had not lodged.

B. Nodule-Damaging Insect Larvae Tests

Several experiments were conducted from 1976 to 1978 to study the effects of nodule damage caused by larvae of several insects including the bean leaf beetle, banded cucumber beetle and the platystomatid fly on $N_2$ fixation by soybeans.

The first experiment was conducted on the Dan Richard farm near Port Barre, La. Adults of each of the insects mentioned above were introduced into small screen cages (1 x 1 x 1 m) placed over one-month-old 'Forrest' soybeans. Treatments were replicated three times. An equal number of screen cages into which no insects were introduced were included in the experiment to serve as controls. The soil in the control cages was treated with carbofuran to kill any insect larvae that might have been present. Plants were excavated in the R2 stage of development on July 13, 1976, and two samples of two plants each from each cage were tested for nitrogenase activity. Uncaged control plants taken from the vicinity of the cages and from approximately 50 m into the field were also included in the study. The nodules on each plant were later examined for nodule injury caused by insect larvae. Interpretation of data in Table 17 indicated that the average number of damaged nodules per plant was lowest in the caged control treatment. Average values for nodule damage were ten, nine and five times greater in the platystomatid fly, bean leaf beetle and banded

Table 17. Effects of several nodule-damaging insects on nodulation and $C_2H_4$ production by soybeans grown in screen cages in the field near Krotz Springs, La. in 1976; means for three replications.

| Treatment[1/] | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|
| | - - - - number | - - - - | - - mg - - | - - - - - - - - - | - µM - - - - - - - | - - - - - |
| Caged Control | 62.3 | 1.3 | 131.6 | 7.61 | 0.1335 | 63.1 |
| *Rivellia quadrifasciata* | 84.6 | 13.6 | 114.7 | 3.53 | 0.0408 | 29.6 |
| Bean leaf beetle | 79.3 | 11.5 | 105.7 | 3.94 | 0.0483 | 41.7 |
| Banded cucumber beetle | 75.6 | 8.2 | 119.8 | 4.17 | 0.0542 | 34.7 |
| Uncaged Control | 67.3 | 5.6 | 118.5 | 4.60 | 0.0683 | 36.7 |
| Random checks from within field | 105.3 | 14.7 | 185.0 | 5.37 | 0.0515 | 29.8 |
| L.S.D. (0.05) | | 8.09 | | 2.65 | 0.0321 | 18.6 |
| L.S.D. (0.01) | | 10.90 | | 3.57 | 0.0432 | 25.1 |

cucumber beetle cages, respectively, than in the control cages. Plants randomly taken from within the field were subjected to as great or greater nodule damage than those in the insect cages. Therefore, it appeared that on the basis of nodule damage, only the caged controls could be considered as true controls. Apparently only the cage had protected these plants from the severe nodule damage inflicted upon the uncaged control plants. It was noted that all of the insects caused significant reductions in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight. It was also noted that the uncaged control plants taken from the vicinity of the cages and the random checks taken from within the field were lower in all parameters of $N_2$ fixation ($C_2H_4$ produced) than the caged controls. The number of damaged nodules on the control plants was significantly lower than on the other plants except for the uncaged controls. No significant differences were detected, however, in nodule dry weight per plant. Apparently the plants that received heavy nodule damage responded by producing new nodules which were smaller in size than those on the caged controls.

Two investigations were conducted during 1977 to study the effects of damage caused by natural populations of bean leaf beetle and platystomatid fly larvae on $N_2$ fixation by soybeans. One investigation was carried out in a field of 'Dare' soybeans on the Dan Richard farm near Krotz Springs, La., and the other was in a field of 'Dare' soybeans on the Bill Pollingue farm near Port Barre, La. At both locations soil applications of carbofuran and aldicarb were used to control the larvae of the platystomatid fly and the bean leaf beetle. Four replications each of insecticide-treated and untreated control plots were included in those studies. Experiments were conducted at both locations on two dates, June 15 and July 13, 1977, to determine the nitrogenase activity for the insecticide-treated and untreated plants. In each of the experiments four samples with three plants each were taken from each plot.

At Krotz Springs and Port Barre very similar results were obtained on both sampling dates. Soil applications of carbofuran and aldicarb caused a significant increase in the average number and dry nodule weight per plant and a significant decrease in number of damaged nodules per plant at both locations (Tables 18 and 19). Significant increases in $N_2$ fixed ($C_2H_4$ produced) per plant were noted in the insecticide-treated plants at both locations on June 15 and July 13, 1977. The June 15 sampling showed that the insecticide-treated plants produced 10 and 12 times more $C_2H_4$ per plant than the untreated controls at Krotz Springs and Port Barre, respectively. The July 13 sampling showed that the insecticide-treated plants produced about twice as much $C_2H_4$ per plant as the untreated controls. Increases were also detected in the amount of $N_2$ fixed ($C_2H_4$ produced) per nodule and per g of dry nodule due to the insecticide treatment. These differences were significant only on June 15 at both locations, however.

Ten plants were randomly selected from each plot at Krotz Springs on June 8, 1976, and the tops were analyzed for total-N. As shown in Table 20, the plant tops in the insecticide-treated plots were

Table 18. Effects of controlling predacious insect larvae on nodulation and $C_2H_4$ production by field-grown soybeans near Krotz Springs, La. during 1977; means for four replications.

| Treatment | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|
| | - - - - number | - - - - | - - mg - - | - - - - - - - - - - | - $\mu$M - | - - - - - - - - - - |
| Treated[1/], June, 15 | 14.1** | | 51.0** | 2.38** | 0.13** | 42.8* |
| July, 13 | 54.8** | 0.23** | 103.3** | 2.84** | 0.05 | 25.8 |
| Control, June, 15 | 8.8 | | 13.67 | 0.23 | 0.03 | 19.1 |
| July, 13 | 34.5 | 5.27 | 40.67 | 1.45 | 0.04 | 74.8 |

*, ** Significantly different from the control at the 5 and 1% level of probability, respectively.

1/ Carbofuran and aldicarb were used to control nodule damaging insect larvae.

Table 19. Effects of controlling predacious insect larvae on nodulation and $C_2H_4$ production by field-grown soybeans near Port Barre, La. during 1977; means for four replications.

| Treatment | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|
| | ---- number ---- | | -- mg -- | ---------- μM ---------- | | |
| Treated[1/], June, 15 | 28.1** | | 101.0** | 3.64** | 0.15** | 37.0* |
| July, 13 | 108.9* | 0.71* | 244.7** | 5.92** | 0.07 | 27.5 |
| Control, June, 15 | 11.1 | | 14.0 | 0.31 | 0.03 | 20.6 |
| July, 13 | 68.6 | 10.59 | 87.7 | 2.46 | 0.04 | 25.4 |

*, ** Significantly different from the control at the 5 and 1% levels of probability, respectively.

1/ Carbofuran and aldicarb were used to control nodule-damaging insect larvae.

significantly higher in percent N than plants taken from the untreated plots. Plants in the control plots had received considerable damage from the soybean thrips <u>Sericothrips variabilis</u> (Beach), but the insecticide-treated plots received no apparent damage from this insect; therefore, it was not determined whether the difference in percent N in the plant tops was a result of thrips damage or the result of damage to the root nodules caused by insect larvae.

On June 25, 1977, 10 plants were taken from each plot at Krotz Springs and Port Barre, and the leaf area of each plant was determined with an area meter. Average leaf area per plant was greater for the insecticide-treated plants at both locations; however, this difference was significant only at Port Barre (Tables 20 and 21). Average seed yield for the insecticide-treated plots was significantly higher than the control plots at both locations. Yields were increased by 29 and 28% at Krotz Springs and Port Barre, respectively.

A final investigation was conducted near St. Gabriel, La., during 1978 to study again the effects of bean leaf beetle larvae on nodulation and $N_2$ fixation ($C_2H_4$ produced) by soybeans and to confirm the data obtained in the previous studies. For the purpose of this study, 'Bragg' and 'Forrest' soybeans were grown in large (16.7 x 16.7 x 2.4 m) screen cages, and various numbers of adult bean leaf beetles were introduced into each cage. Four of the cages were infested with bean leaf beetles, and a fifth cage was used as an undamaged control.

On July 12, 1978, five plant samples composed of three plants each were taken from each cage for the $C_2H_2$ reduction assay. In this study only the 'Forrest' cultivar was sampled. The plant tops were

Table 20. Effects of controlling predacious insect larvae on N content, growth and yield of field-grown soybeans near Krotz Springs, La. during 1977; means for four replications.

| Treatment | Total N in tops | Leaf area per plant | Seed Yield |
|---|---|---|---|
| | -%- | -$cm^2$- | -kg/ha- |
| Treated[1/] | 3.41** | 671.2 | 1998** |
| Control | 3.00 | 528.8 | 1412 |

*, ** Significantly different from the control at the 5 and 1% levels of probability, respectively.

1/ Carbofuran and aldicarb were used to control nodule-damaging insect larvae.

Table 21. Effects of controlling predacious insect larvae on growth and yield of field-grown soybeans near Port Barre, La. during 1977; means for four replications.

| Treatment | Leaf area per plant | Seed Yield |
|---|---|---|
| | -$cm^2$- | -kg/ha- |
| Treated[1/] | 1702.3** | 2551** |
| Control | 841.0 | 1831 |

** Significantly different from the control at the 1% level of probability.

1/ Carbofuran and aldicarb were used to control nodule-damaging insect larvae.

saved and analyzed for total-N. Compared to the control plants, the average number of damaged nodules per plant was significantly higher on the plants that were grown in all of the cages that had been infested with bean leaf beetles (Table 22). No significant differences were noted for any of the other parameters.

On August 17, 1978, the 'Forrest' and 'Bragg' soybeans were sampled for the $C_2H_2$ reduction assay. For this study 10 plants of each variety were taken from each cage. As shown in Table 23, infestation of the plots caused no significant differences in the average number of nodules per plant for the 'Forrest' cultivar, although the values for the beetle-infested cages were all lower than the control. No significant differences in number of damaged nodules per plant were detected; however, the values for the insect-infested plants were all greater than the control. With respect to nodule dry weight per plant, the three highest levels of bean leaf beetle infestation were significantly decreased as compared to the control. This indicated that in order to compensate for nodules which were destroyed by bean leaf beetle larvae, the infested plants produced new nodules which were smaller than those on the control plants. The amount of $N_2$ fixed ($C_2H_4$ produced) per plant by the insect-infested plants reflected the harmful reduction in nodule dry weight. Values for this parameter were significantly reduced in two of the insect-damaged cages. Similar results were obtained for $N_2$ fixed ($C_2H_4$ produced) per nodule. Plants taken from the three most heavily infested cages were significantly lower in $N_2$ fixed ($C_2H_4$ produced) per nodule than the control plants. No significant differences were detected in $N_2$ fixed ($C_2H_4$ produced)

Table 22. Effects of bean leaf beetle larvae damage on growth, nodulation and $C_2H_4$ production by 'Forrest' soybeans grown in screen cages; means for five replications.

| Number of adult insects per cage1/ | Leaf area per plant | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | - - - - number | - - - - | - mg - | - - - - - - - - - - $\mu$M | - - - - - - - - - - | |
| None | 1383.5 | 14.3 | 0.6 | 33.3 | 0.33 | 0.0248 | 9.9 |
| 1050 | 1444.4 | 13.6 | 6.7 | 34.0 | 0.64 | 0.0441 | 17.00 |
| 1700 | 1565.2 | 19.2 | 10.5 | 36.0 | 0.68 | 0.0323 | 16.5 |
| 4200 | 1642.8 | 18.3 | 11.1 | 33.0 | 0.41 | 0.0245 | 11.7 |
| 8340 | 1265.3 | 11.5 | 7.5 | 24.0 | 0.21 | 0.0195 | 8.5 |
| L.S.D. (0.05) | | | 3.7 | | | | |
| L.S.D. (0.01) | | | 5.1 | | | | |

1/ Adult bean leaf beetles were put in the cages on June 20, 1978 and July 10, 1978.

Table 23. Effects of bean leaf beetle larvae damage on growth, nodulation and $C_2H_4$ production by 'Forrest' soybeans grown in screen cages; means for 10 replications.

| Number of adult insects per cage[1/] | Leaf area per plant | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|---|
| | $cm^2$ | number | | mg | uM | | |
| None | 2395.3 | 60.6 | 9.7 | 213.0 | 8.90 | 0.1521 | 43.4 |
| 1050 | 1769.1 | 56.6 | 22.3 | 152.0 | 6.06 | 0.1091 | 37.7 |
| 1700 | 1609.3 | 51.9 | 19.7 | 91.0 | 3.71 | 0.0804 | 63.5 |
| 4200 | 2105.5 | 27.7 | 15.6 | 36.0 | 1.33 | 0.0423 | 35.3 |
| 8340 | 1702.3 | 56.1 | 19.0 | 128.0 | 6.81 | 0.0970 | 45.5 |
| L.S.D. (0.05) | | | | 85.2 | 4.27 | 0.0526 | |
| L.S.D. (0.01) | | | | 113.5 | 5.69 | 0.0701 | |

1/ Adult bean leaf beetles were put in the cages on June 20, 1978 and July 10, 1978.

per g of dry nodule or in average leaf area per plant.

The data for the 'Bragg' soybeans sampled on the same date (August 17, 1978) are shown in Table 24. In this study no significant differences were detected for any of the parameters.

Both the 'Bragg' and 'Forrest' cultivars were sampled again on September 12, 1978. Data pertaining to this sampling are presented in Tables 25 and 26, respectively. Interpretation of the data for the 'Forrest' cultivar indicated that none of the values were significantly lower than the control values. Similar results were obtained with the 'Bragg' cultivar; however, in this study $N_2$ fixed ($C_2H_4$ produced) per g of dry nodule was significantly lower in the plants with the highest level of bean leaf beetle infestation as compared to the control plants. Probably due to the late stage of development (R5), $N_2$ fixation ($C_2H_4$ produced) by all the samples was very low on September 12, 1978. This was also true for the 'Forrest' variety which was in the R6 stage of development. It was noted that nodule damage in the control cage was as great or greater than the nodule damage in the bean leaf beetle-infested cages. Sufficient numbers of bean leaf beetles gained access to the control cage after mid July to cause significant nodule damage to these plants. This may explain why no differences in $N_2$ fixation ($C_2H_4$ produced) were detected in several of the studies.

Data from these field investigations indicated that nodule-damaging insect larvae such as those of the bean leaf beetle and platystomatid fly destroy nodules and cause significant reductions of $N_2$ fixation ($C_2H_4$ produced) by soybeans. Studies also indicated that in some soybean fields in Louisiana, these insects often reach

Table 24. Effects of bean leaf beetle larvae damage on growth, nodulation and $C_2H_4$ production by 'Bragg' soybeans grown in screen cages; means for 10 replications.

| Number of adult insects per cage[1/] | Leaf area per plant | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|---|
| | -$cm^2$- | - - - - number | - - - - | - mg - | - - - - - - - - - - | $\mu$M - - - - - - - - - | |
| None | 3947.3 | 79.2 | 15.6 | 281.0 | 9.10 | 0.1047 | 29.8 |
| 1050 | 3214.5 | 88.7 | 37.4 | 197.0 | 6.78 | 0.0757 | 40.3 |
| 1700 | 3119.9 | 155.7 | 35.3 | 267.0 | 11.43 | 0.0709 | 47.0 |
| 4200 | 4396.6 | 119.3 | 43.7 | 232.0 | 7.44 | 0.0636 | 30.3 |
| 8340 | 2539.8 | 88.3 | 29.5 | 106.0 | 3.95 | 0.0490 | 41.5 |
| F test | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |

1/ Adult bean leaf beetles were put in the cages on June 20, 1978 and July 10, 1978.

N.S. Not significant at the 5% level of probability.

Table 25. Effects of bean leaf beetle larvae damage on growth, nodulation and $C_2H_4$ production by 'Forrest' soybeans grown in screen cages; means for five replications.

| Number of adult insects per cage1/ | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|
| | ---- number | ---- | - mg - | ---------- | $\mu$M | ---------- |
| None | 23.2 | 6.2 | 159 | 1.26 | 0.0485 | 7.6 |
| 1050 | 20.2 | 6.4 | 152 | 0.44 | 0.0210 | 3.1 |
| 1700 | 15.2 | 5.2 | 114 | 0.22 | 0.0183 | 2.2 |
| 4200 | 13.2 | 3.4 | 90 | 1.04 | 0.0916 | 14.5 |
| 8340 | 12.8 | 3.0 | 83 | 0.23 | 0.0174 | 3.9 |
| L.S.D. (0.05) | | | | | 0.0391 | 6.4 |
| L.S.D. (0.01) | | | | | 0.0533 | 8.7 |

1/ Adult bean leaf beetles were put in the cages on June 20, 1978 and July 10, 1978.

Table 26. Effects of bean leaf beetle larvae damage on growth, nodulation and $C_2H_4$ production by 'Bragg' soybeans grown in screen cages; means for five replications.

| Number of adult insects per cage[1/] | Nodules per plant | Damaged nodules per plant | Nodule dry weight per plant | $C_2H_4$ per plant | $C_2H_4$ per nodule | $C_2H_4$ per g of dry nodule |
|---|---|---|---|---|---|---|
| | - - - - number | - - - - | - mg - | - - - - - - - - - - | - $\mu$M - - - - - | - - - - - - |
| None | 42.8 | 11.2 | 217.8 | 3.11 | 0.0692 | 14.3 |
| 1050 | 37.2 | 7.4 | 199.5 | 2.06 | 0.0684 | 11.5 |
| 1700 | 49.4 | 12.0 | 213.3 | 1.04 | 0.0270 | 6.9 |
| 4200 | 43.4 | 9.8 | 200.0 | 1.80 | 0.0427 | 11.0 |
| 8340 | 45.8 | 14.8 | 159.9 | 0.47 | 0.0097 | 3.0 |
| L.S.D. (0.05) | | | | | | 7.3 |

1/ Adult bean leaf beetles were put in the cages on June 20, 1978 and July 10, 1978.

population levels that are adequate to significantly reduce $N_2$ fixation ($C_2H_4$ produced) and seed yield of soybeans.

## III. RECOVERY OF RHIZOBIUM JAPONICUM, STRAIN 110, IN SOYBEAN NODULES

A three-year investigation was conducted during 1976, 1977 and 1978, at the Burden Research Center in Baton Rouge, La., to study the effectiveness of seed inoculation with a non-indigenous strain of *Rhizobium japonicum*, strain 110, in overcoming indigenous populations of *R. japonicum* to form root nodules on field-grown 'Lee' soybeans. Uninoculated 'Lee' soybeans were included in these studies so that the effects of seed inoculation with *R. japoncium*, strain 110, and Nitragin® peat-base inoculant on nodulation, $N_2$ fixation ($C_2H_4$ produced) and seed yield could be studied. Also, a non-nodulating isoline of 'Lee' soybeans was included so that estimates of the contributions of $N_2$ to the seeds of nodulated soybeans could be made.

The treatments used in this three-year study are listed and described briefly below:

Treatment 1. Nodulating 'Lee' soybeans were planted without inoculation, and no fertilizer was applied (Control).

Treatment 2. Nodulating 'Lee' soybeans were inoculated with $10^4$ *R. japonicum*, strain 110, cells per cm of row, and no fertilizer N was applied (N-$10^4$).

Treatment 3. Nodulating 'Lee' soybeans were inoculated with $10^8$ *R. japonicum*, strain 110, cells per cm of row, and no fertilizer N was applied (N-$10^8$)

Treatment 4. Nodulating 'Lee' soybeans were inoculated with peat-base inoculant, and no fertilizer N was applied (N-P).

Treatment 5. Nodulating 'Lee' soybeans were inoculated with peat-base inoculant and were fertilized with the equivalent of 250 kg of $NH_4N_3$-N per ha applied in five split applications (N-P-250).

Treatment 6. Non-nodulating 'Lee' soybeans were grown with no supplemental N fertilization (NN-0).

Treatment 7. Non-nodulating 'Lee' soybeans were fertilized with the equivalent of 250 kg of $NH_4NO_3$-N per ha applied in five split applications (NN-250).

Data for the average number of nodules per plant, dry weight of nodules per plant and recovery of *R. japonicum*, serogroup 110, from the nodules in the first year of the study (1976) are presented in Table 27. No significant differences were detected between the treatments in number of nodules per plant; however, significant differences were noted in the average dry weight of nodules per plant. The nodulating soybeans inoculated with peat-base inoculant and fertilized with 250 kg of N per ha (N-P-250) produced significantly less nodule dry weight per plant than the unfertilized peat-inoculated plants (N-P) and the uninoculated, unfertilized plants (controls). This could have resulted from the supplemental supply of fertilizer-N. However, plants that received inoculation with *R. japonicum*, strain 110, (N-$10^4$ and N-$10^8$) also produced significantly less nodule dry weight per plant than the control plants. Recovery of *R. japonicum*, serogroup 110, from the root nodules was very low in both of the treatments inoculated with *R. japonicum*, strain 110, (N-$10^4$ and N-$10^8$). Serological studies of the nodules produced on these plants resulted in only five positive tests for serogroup 110 out of a possible 144, or 3.5% recovery of serogroup 110.

Results of the $C_2H_2$ reduction assay and plant height measurements for each of the treatments are given in Table 28. Among the nodulating soybean treatments, no significant differences were found to exist in $N_2$ fixed ($C_2H_4$ produced) per plant or per g of dry nodule weight. The N-P-250 treatment was significantly lower in $N_2$ fixed ($C_2H_4$ produced) per nodule than the N-P treatment and the control.

Plant heights for the non-nodulating 'Lee' soybeans (NN-0 and NN-250) were found to be significantly greater than for the nodulated soybeans. For this reason it was decided that the non-nodulating soybean isoline used in this study was not a true isoline of 'Lee'. Therefore, a new lot of non-nodulating 'Lee' soybeans was obtained for use in 1977 and 1978.

The non-nodulating soybeans that received no N fertilization (NN-0) were significantly lower in seed yield than all of the other treatments (Table 29). When these soybeans were fertilized with the equivalent of 250 kg of N per ha (NN-250), yields remained significantly lower than several of the treatments including the control. The NN-0 and NN-250 treatments were also significantly lower than the control with respect to percent N in the seeds. The percent protein in the seeds was estimated by multiplying percent N by 6.25. These values ranged from 40.9% protein in the control and N-$10^4$ treatments to only 31% in the NN-0 treatment. The contribution of symbiotically fixed $N_2$ in the seeds of the nodulated soybeans was obtained by subtracting the N content (kg/ha) in the seeds of the NN-0 plants from the N content (kg/ha) in the seeds of the unfertilized, nodulated soybeans. These values ranged from 127.1 kg/ha for the N-$10^8$ treatment to 89.6 kg/ha for the N-P treatment.

Table 27. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on nodulation and recovery of serogroup 110 in nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1976; means for six replications.

| Treatment | Nodules per plant | Nodule dry wt. per plant | Recovery of *R. japonicum* serogroup 110, in nodules |
|---|---|---|---|
| | -number- | -mg- | -%- |
| Control1/ | 79.3 | 248.0 | - |
| N-$10^8$ 2/ | 87.0 | 199.3 | 3.5 |
| N-$10^4$ 3/ | 74.0 | 169.3 | 3.5 |
| N-P4/ | 93.3 | 290.0 | - |
| N-P-250 5/ | 53.0 | 103.3 | - |
| L.S.D. (0.05) | | 47.2 | |
| L.S.D. (0.01) | | 64.0 | |

1/ Nodulating 'Lee' soybeans with no inoculation with *R. japonicum*.
2/ Nodulating 'Lee' soybeans inoculated with $10^8$ *R. japonicum*, strain 110, per cm of row.
3/ Nodulating 'Lee' soybeans inoculated with $10^4$ *R. japonicum*, strain 110, per cm of row.
4/ Nodulating 'Lee' soybeans inoculated with peat-base inoculant.
5/ Nodulating 'Lee' soybeans inoculated with peat-base inoculant and fertilized with 250 kg of $NH_4NO_3$-N per ha.

Table 28. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on $C_2H_4$ production and plant growth of nodulating and non-nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1976; means for six replications.

| Treatment | $C_2H_4$ per plant | $C_2H_4$ per nodule $\times 10^{-2}$ | $C_2H_4$ per g of dry nodule | Plant height |
|---|---|---|---|---|
| | - - - - - - - - - - - - - - | $\mu$M - - - - - - - - - - - - - - | | -cm- |
| Control | 4.73 | 5.66 | 18.6 | 87.4 |
| N-$10^8$ | 5.50 | 6.22 | 17.6 | 94.9 |
| N-$10^4$ | 3.74 | 4.84 | 21.3 | 90.1 |
| N-P | 5.27 | 4.99 | 16.4 | 82.3 |
| N-P-250 | 1.39 | 2.53 | 12.2 | 94.7 |
| NN-0[1/] | 0.00 | -- | -- | 117.5 |
| NN-250[2/] | 0.00 | -- | -- | 107.6 |
| L.S.D. (0.05) | | 2.14 | | 19.1 |
| L.S.D. (0.01) | | 2.90 | | 25.7 |

1/ Non-nodulating 'Lee' soybeans with no N fertilization.

2/ Non-nodulating 'Lee' soybeans with 250 kg of $NH_4NO_3$-N per ha.

Various nodule data collected the second year of the study (1977) were very similar to those obtained in 1976 (Table 30). Nodule number and nodule dry weight per plant were significantly lower in the N-P-250 treatment than in the control. Again, recovery of <u>R. japonicum</u>, serogroup 110, from the root nodules was low with only 7.6 and 3.5% recovery in the N-$10^4$ and N-$10^8$ treatments, respectively.

Results of the $C_2H_2$ reduction assay are presented in Table 31. $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight was significantly lower in the N-P-250 and N-P treatment than in the control. The N-$10^4$ treatment was found to be significantly lower in $N_2$ fixed ($C_2H_4$ produced) per nodule than the control. The average fresh weight, dry weight and N content of the plant tops, collected at the time the $C_2H_2$ reduction assay was performed, are presented in Table 31. Four of the treatments (N-P, N-P-250, NN-O and NN-250) were found to have significantly lower fresh and dry plant top weights than the control. No significant differences were detected between any of the treatments in percent N of the plant tops.

The average seed yield of the N-P-250, NN-O and NN-250 treatments were significantly lower than the control (Table 32). The percent N in the seeds was significantly lower in the NN-O and NN-250 treatments than in the control. The contribution of seed N attributed to $N_2$ fixation was estimated to range from 75.6 kg/ha in the N-$10^8$ treatment to 79.7 kg/ha in the control.

In the third year of the study (1978), the average number of nodules per plant as well as nodule fresh and dry weight per plant was reduced in the N-$10^8$ and N-$10^8$-250 treatments as compared to the control (Table 33). It is believed that this occurrence was a result of having

Table 29. Effects of N fertilization and seed inoculation with <u>Rhizobium japonicum</u>, strain 110, or peat-base inoculant on yield and N content of nodulating and non-nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1976; means for six replications.

| Treatment | Seed Yield | N in seed | Protein in seed | Seed N | Symbiotically fixed N in seed |
|---|---|---|---|---|---|
| | - kg/ha - | -%- | -%- | - - - - - - kg/ha | - - - - - - |
| Control | 2217.6 | 6.54 | 40.9 | 145.0 | 117.7 |
| N-$10^8$ | 2372.2 | 6.51 | 40.7 | 154.4 | 127.1 |
| N-$10^4$ | 2130.2 | 6.54 | 40.9 | 139.3 | 112.0 |
| N-P | 1888.3 | 6.19 | 38.7 | 116.9 | 89.6 |
| N-P-250 | 2237.8 | 6.15 | 38.4 | 137.6 | --- |
| NN-O | 551.0 | 4.96 | 31.0 | 27.3 | --- |
| NN-250 | 1827.8 | 5.47 | 34.2 | 100.0 | --- |
| L.S.D. (0.05) | 366.2 | 1.00 | | | |
| L.S.D. (0.01) | 491.9 | 1.34 | | | |

Table 30. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on nodulation and recovery of serogroup 110 in nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1977; means for six replications.

| Treatment | Nodules per plant | Nodule dry wt. per plant | Recovery of *R. japonicum* serogroup 110, in nodules |
|---|---|---|---|
| | -number- | -mg- | -%- |
| Control | 16.9 | 22.7 | - |
| N-$10^8$ | 19.4 | 25.0 | 3.5 |
| N-$10^4$ | 22.0 | 23.0 | 7.6 |
| N-P | 14.3 | 13.7 | - |
| N-P-250 | 5.8 | 5.7 | - |
| L.S.D. (0.05) | 6.9 | 8.5 | |
| L.S.D. (0.01) | 9.2 | 11.3 | |

to replant these two treatments approximately two weeks after the other treatments had been planted. Replanting was deemed necessary because of the poor stands that had been obtained. No explanation can be made for the failure to obtain an acceptable stand in the first planting. Nodule number per plant was not decreased in any of the other treatments, however. Nodule fresh weight per plant was lower in the N-P-250 as compared to the control. Recovery of applied R. japonicum, strain 110, from the root nodules was slightly higher in 1978 than in previous years with 9.7 and 6.9% of the nodules on the N-$10^8$ and N-$10^4$ plants, respectively, belonging to serogroup 110. The application of combined N seemed to reduce recovery of serogroup 110 in the N-$10^8$-250 treatment as compared to the N-$10^8$ treatment. The N-$10^8$-250 treatment was added to the study in 1978 and consisted of nodulating 'Lee' soybeans inoculated with $10^8$ R. japonicum, strain 110, cells per cm of row and fertilized with 250 kg of $NH_4NO_3$-N per ha.

The quantity of $N_2$ fixed ($C_2H_4$ produced) per plant and per nodule by the N-$10^8$-250 treatment was found to be significantly lower than the control (Table 34). Also $C_2H_4$ produced per plant was significantly lower in the N-$10^8$ treatment, possibly as a result of replanting. It is interesting to note that for this treatment, $C_2H_4$ produced per g of dry nodule weight was significantly greater than the control. Again, this was attributed to the great decrease in nodule weight per plant rather than an actual increase in $N_2$ fixation ($C_2H_4$ production).

Fresh and dry weight of plant tops produced per plant at the R2 stage of development was significantly lower in the N-$10^8$ and N-$10^8$-250 treatments than in the control. This was possibly due to replanting. Both of the non-nodulating treatments were also lower than the control

Table 31. Effects of N fertilization and seed inoculation with Rhizobium japonicum, strain 110, or peat-base inoculant on $C_2H_4$ production, growth and N content of nodulating and non-nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1977; means for six replications.

| Treatment | $C_2H_4$ per plant | $C_2H_4$ per nodule x $10^{-2}$ | $C_2H_4$ per g of dry nodule | Fresh weight of tops per plant | Dry weight of tops per plant | N in tops |
|---|---|---|---|---|---|---|
| | - - - - - - - - - - - | $\mu$M - - - - - - - - - - - | | - - - - - - - - g | - - - - - - - - | -%- |
| Control | 0.49 | 3.34 | 21.9 | 47.49 | 12.67 | 2.85 |
| N-$10^8$ | 0.55 | 2.58 | 21.1 | 47.75 | 12.52 | 2.51 |
| N-$10^4$ | 0.58 | 2.23 | 20.5 | 44.35 | 11.40 | 2.53 |
| N-P | 0.16 | 1.21 | 11.9 | 32.67 | 8.77 | 3.01 |
| N-P-250 | 0.02 | 0.41 | 4.6 | 26.88 | 7.51 | 2.91 |
| NN-0 | 0.00 | -- | -- | 24.62 | 6.97 | 2.24 |
| NN-250 | 0.00 | -- | -- | 24.76 | 6.81 | 2.43 |
| L.S.D. (0.05) | 0.28 | 0.94 | 8.2 | 12.58 | 3.49 | |
| L.S.D. (0.01) | 0.37 | 1.25 | 10.9 | 16.72 | 4.10 | |

Table 32. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on yield and N content of nodulating and non-nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1977; means for six replications.

| Treatment | Seed Yield | N in seed | Protein in seed | Seed N | Symbiotically fixed N in seed |
|---|---|---|---|---|---|
| | - kg/ha - | -%- | -%- | - - - - - - kg/ha | - - - - - - |
| Control | 1541.9 | 6.38 | 39.9 | 98.4 | 79.7 |
| N-$10^8$ | 1478.0 | 6.38 | 39.9 | 94.3 | 75.6 |
| N-$10^4$ | 1576.5 | 6.28 | 39.3 | 99.0 | 80.3 |
| N-P | 1484.7 | 6.50 | 40.6 | 96.5 | 77.8 |
| N-P-250 | 1229.5 | 6.41 | 40.0 | 78.8 | -- |
| NN-O | 415.9 | 4.50 | 28.1 | 18.7 | -- |
| NN-250 | 1216.5 | 5.65 | 35.3 | 68.7 | -- |
| L.S.D. (0.05) | 191.3 | 0.53 | | | |
| L.S.D. (0.01) | 255.7 | 0.71 | | | |

Table 33. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on nodulation and recovery of serogroup 110 in nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1978; means for six replications.

| Treatment | Nodules per plant | Nodule fresh wt. per plant | Nodule dry wt. per plant | Recovery of *R. japonicum* serogroup 110, in nodules |
|---|---|---|---|---|
| | -number- | - - - - - - - mg | - - - - - - - | -%- |
| Control | 75.3 | 648.7 | 206.3 | - |
| N-$10^8$ | 41.1 | 325.7 | 96.3 | 9.7 |
| N-$10^8$-250 [1/] | 28.5 | 85.7 | 38.3 | 5.6 |
| N-$10^4$ | 83.5 | 674.0 | 202.0 | 6.9 |
| N-P | 64.9 | 548.7 | 161.3 | - |
| N-P-250 | 59.2 | 347.0 | 123.7 | - |
| L.S.D. (0.05) | 32.5 | 296.3 | 84.3 | |
| L.S.D. (0.01) | 43.7 | 399.0 | 113.7 | |

1/ Nodulating 'Lee' soybeans inoculated with $10^8$ *R. japonicum*, strain 110, per cm of row and fertilized with 250 kg of $NH_4NO_3$-N per ha.

Table 34. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on $C_2H_4$ production, growth and N content of nodulating and non-nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1978; means for six replications.

| Treatment | $C_2H_4$ per plant | $C_2H_4$ per nodule x $10^{-2}$ | $C_2H_4$ per g of dry nodule | Fresh weight of tops per plant | Dry weight of tops per plant | N in tops |
|---|---|---|---|---|---|---|
| | ------------ μM ------------ | | | -------- g -------- | | -%- |
| Control | 6.13 | 8.8 | 30.5 | 92.2 | 24.1 | 2.84 |
| N-$10^8$ | 2.97 | 8.2 | 66.1 | 50.6 | 13.4 | 3.66 |
| N-$10^8$-250 | 0.57 | 1.9 | 13.2 | 57.8 | 15.7 | 3.74 |
| N-$10^4$ | 8.37 | 10.5 | 44.0 | 99.6 | 25.0 | 3.01 |
| N-P | 6.60 | 8.7 | 43.7 | 71.1 | 19.5 | 3.14 |
| N-P-250 | 1.40 | 3.0 | 13.0 | 102.5 | 26.6 | 3.60 |
| NN-0 | -- | -- | -- | 53.0 | 14.2 | 2.22 |
| NN-250 | -- | -- | -- | 61.1 | 16.2 | 2.60 |
| L.S.D. (0.05) | 2.87 | 5.5 | 35.0 | 29.0 | 7.5 | 0.60 |
| L.S.D. (0.01) | 3.87 | 7.4 | 47.1 | 38.9 | 10.0 | 0.82 |

in fresh and dry weight of plant tops. Percent N in the plant tops was significantly higher than the control in the N-P-250 and N-$10^8$-250 and N-P treatments. Only one treatment, NN-0, was significantly lower than the control in percent N in the plant tops.

Table 35 contains data on seed yield and N content of the seeds produced in each treatment. Seed yield was significantly lower than the control in the N-$10^8$, N-P and NN-0 treatments. The reduced yield in the N-$10^8$ treatment was believed to be due to replanting; however, no explanation can be given for the yield decrease in the N-P treatment. In addition to the lower yield in the NN-0 treatment, the percent N in the seeds was also significantly lower than the control. Values for yield and percent N in the seeds of the NN-250 and N-P-250 treatments were not significantly different from the control values.

Approximately 63 to 148 kg/ha of symbiotically fixed $N_2$ was present in the seeds of the unfertilized, nodulated soybean plants.

A summary of findings for this three-year investigation are as follows:

1. Seed inoculation with R. japonicum, strain 110, at rates as high as $10^8$ cells per cm of row demonstrated that the applied rhizobia were not very effective in competing with the indigenous rhizobia for nodule sites. Rates of recovery in the nodules during the three-year study ranged from 3.5 to 9.7%.

2. Seed inoculation with R. japonicum, strain 110, or peat-base inoculant did not significantly influence nodulation, $N_2$ fixation ($C_2H_4$ production), N content of the plant tops, percent N in the seeds or seed yield.

Table 35. Effects of N fertilization and seed inoculation with *Rhizobium japonicum*, strain 110, or peat-base inoculant on yield and seed N content of nodulating and non-nodulating 'Lee' soybeans grown at the Burden Research Center, Baton Rouge, La. in 1978; means for six replications.

| Treatment | Seed Yield | N in seed | Protein in seed | Seed N | Symbiotically fixed N in seed |
|---|---|---|---|---|---|
| | - kg/ha - | -%- | -%- | - - - - - - kg/ha | - - - - - - |
| Control | 1879 | 8.47 | 52.9 | 159.2 | 128.9 |
| $N-10^8$ | 1162 | 8.07 | 50.4 | 93.8 | 63.5 |
| $N-10^8-250$ | 1525 | 8.94 | 55.9 | 136.3 | --- |
| $N-10^4$ | 2010 | 8.88 | 55.5 | 178.5 | 148.2 |
| N-P | 1434 | 9.54 | 59.6 | 136.8 | 106.5 |
| N-P-250 | 1687 | 8.67 | 54.2 | 146.3 | --- |
| NN-O | 485 | 6.24 | 39.0 | 30.3 | --- |
| NN-250 | 1737 | 7.97 | 49.8 | 138.4 | --- |
| L.S.D. (0.05) | 419.4 | 1.01 | 6.31 | | |
| L.S.D. (0.01) | 561.1 | 1.37 | 8.56 | | |

3. The equivalent of 250 kg of $NH_4NO_3$-N applied in five equal applications was sufficient to reduce the number and dry weight of nodules per plant and decrease $N_2$ fixation ($C_2H_4$ production) by soybeans.

4. Neither seed yield nor seed N content was increased by the application of 250 kg of $NH_4NO_3$-N per ha.

5. Two out of three years, 250 kg of $NH_4NO_3$-N increased yields of the non-nodulating soybeans to levels that were not significantly different from the nodulated controls.

6. The estimated contribution of symbiotically fixed $N_2$ to the seeds of the nodulated soybeans ranged from 63.5 kg/ha to 128.9 kg/ha.

# SUMMARY AND CONCLUSIONS

Greenhouse studies were conducted to study the effects of various types of insect injury on the $N_2$-fixing system of soybeans. Interpretation of the data collected in these studies indicated that each type of insect injury that was investigated resulted in significant reductions in $N_2$ fixation as measured by the acetylene reduction assay. In many of these studies the number and dry weight of nodules, as well as plant growth, were adversely affected. Several studies provided conclusive evidence that significant reductions in the amount of $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule weight resulted when the leaves of soybean plants were damaged by insects such as the soybean looper. These reductions in $N_2$ fixation ($C_2H_4$ production) were noted as soon as one day after the leaves had been damaged.

Similar reductions in $N_2$ fixation ($C_2H_4$ production) were also detected when some of the leaves were clipped from the plant by hand and when holes were punched in the leaves with a hole puncher. The experimental data did not prove conclusively whether these two methods of mechanical defoliation were valid means of simulating insect-induced defoliation of soybeans.

$N_2$ fixation ($C_2H_4$ production) resulting from damage to the leaf tissue was thought to be due to the decreased supply of photosynthate available for translocation to the root nodules. This photosynthate is necessary to fuel the $N_2$ fixation process.

In another greenhouse study, damage to soybean stems inflicted by the southern green stink bug resulted in a significant decrease in number and dry weight of nodules produced on the injured plants. Significant reductions in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule were also noted. The reduction in $N_2$ fixation ($C_2H_4$ production) caused by this insect was attributed to the removal of photosynthate from the plant stem.

Several greenhouse and field investigations provided very strong evidence that stem girdling caused by the three-cornered alfalfa hopper resulted in significant reductions in nodule number per plant, nodule dry weight per plant and $N_2$ fixation ($C_2H_4$ production) by soybeans. These studies also demonstrated that the reductions in $N_2$ fixation ($C_2H_4$ production) and nodulation were further decreased when the plants were lodged due to stem weakening in the girdled area. Lodging often occurs in the fields when girdled plants are subjected to high winds. This suggested that yields of soybeans may be significantly reduced, especially if the plants lodged after the pod-filling period began. At this time the adjacent plants would be unable to completely compensate for the loss of the damaged plant. Decreases in nodulation and $N_2$ fixation ($C_2H_4$ production) associated with stem girdling caused by the three-cornered alfalfa hopper was attributed to a reduction in the supply of photosynthate to the nodules of the injured plants. The restricted movement of photosynthate through the girdled region of the stem also resulted in more rapid stem growth above this area than below. As a result, the stems were noticeably larger in diameter above the injured area.

Data obtained in extensive field studies during 1976, 1977 and 1978 indicated that naturally existing populations of insect larvae such as those of the banded cucumber beetle, bean leaf beetle and the platystomatid fly cause significant reductions in $N_2$ fixation ($C_2H_4$ production) and yield of soybeans by attacking the nodule and consuming its contents. In field studies conducted near Krotz Springs and Port Barre, La., carbofuran and aldicarb were used successfully to control natural soil-borne populations of these predacious larvae. As a result, the number of damaged nodules found on the insecticide treated plants was significantly reduced. The decrease in nodule damage was associated with significant increases in $N_2$ fixed ($C_2H_4$ produced) per plant, per nodule and per g of dry nodule at Krotz Springs and Port Barre, La. The amount of $C_2H_4$ produced per plant was increased by as much as 92%. Seeds yields were increased in the insecticide treated plots by 29 and 28% at Krotz Springs and Port Barre, respectively.

Investigations were conducted during 1976, 1977 and 1978 to determine the rate of recovery of a non-indigenous strain of *Rhizobium japonicum* (strain 110) in the nodules of 'Lee' soybeans when this strain was banded in contact with the seed at rates of $10^4$ and $10^8$ rhizobia per cm of row. The soil selected for this study contained approximately $10^5$ *R. japonicum* per g of soil. Serological analysis of nodules taken from this field prior to the study indicated that serogroup 110 was not present. During the three year period, recovery of serogroup 110 from the nodules ranged from 3.5 to 9.7%. Seed inoculation with strain 110 or peat-base inoculant did not increase nodulation, $N_2$ fixation ($C_2H_4$ production), seed yield or percent N in the seeds as compared to the uninoculated control.

Another objective of this study was to determine the effects of fertilizing nodulating and non-nodulating soybeans with the equivalent of 250 kg of $NH_4NO_3$-N per ha. The fertilizer was applied in five equal applications of 50 kg/ha of N applied at two week intervals during the growing season. It was hoped that the split applications of N would not adversely affect nodulation and $N_2$ fixation ($C_2H_4$ production). The results of this study, however, indicated that both of these important processes were significantly reduced. The data also indicated that the yields of the fertilized nodulating soybeans were not increased above the levels of the unfertilized nodulating soybeans. Seed yields and seed N content of the unfertilized non-nodulating soybeans averaged only 26 and 74% of the unfertilized nodulating soybeans, illustrating the value of symbiotic $N_2$ fixation. The application of 250 kg of $NH_4NO_3$-N to the non-nodulating soybeans was sufficient to increase the seed yields to levels that were not significantly different from the unfertilized nodulating soybeans; however, the N content of the seeds remained significantly lower. These data were interpreted to indicate that 250 kg/ha of $NH_4NO_3$-N was not able to substitute completely for symbiotically fixed $N_2$.

With the use of mathematical calculations it was estimated that the contribution of symbiotically fixed $N_2$ to the seeds of the nodulating soybeans ranged from 63.5 to 128.9 kg/ha.

## LITERATURE CITED


Abel, G. H. and L. W. Erdman. 1964. Response of Lee soybeans to different strains of Rhizobium japonicum. Agron. J. 56:423-424.

Ahmed, S. and H. J. Evans, 1961. The essentiality of Cobalt for soybean plants grown under symbiotic conditions. Proc. Natl. Acad. Sci. U.S.A. 47:24.

Allos, H. F. and W. V. Bartholomew, 1955. Effect of available N on symbiotic fixation. Soil Sci. Soc. Amer. Proc. 19:182-184.

Allen, O. N. and E. K. Allen. 1940. Response of the peanut plant to inoculation with rhizobia, with special reference to morphological development of the nodules. Bot. Gaz. 102:121-142.

Bach, M. K., W. E. Magee and R. H. Burris, 1958. Translocation of photosynthetic products to soybean nodules and their role in nitrogen fixation. Plant Physiol. 33:118-124.

Begum, A. and W. G. Eden. 1965. Influence of defoliation on yield and quality of soybeans. J. Econ. Entomol. 58:591-592.

Beiberdorf, F. W. 1938. The cytology and histology of the root nodules of some leguminoseae. J. Amer. Soc. Agron. 30:375-389.

Bergersen, F. J. 1971. Biochemistry of symbiotic nitrogen fixation in legumes. Annu. Rev. Plant Physiol. 22:121-140.

Bergersen, F. J. 1963. Iron in the developing soybean nodule. Aust. J. Biol. Sci. 16:916-919.

Bremner, J. M. 1965. Total nitrogen. In C. A. Black, et al. (ed.). Methods of soil analysis. Agron. 9:1149-1178. Am. Soc. of Agron., Madison, Wis.

Brill, W. J., P. E. Bishop and H. J. Evans. 1975. Increasing input of biologically fixed $N_2$ into legumes. p. 37-43. In H. J. Evans (ed.). Enhancing biological nitrogen fixation.

Burley, J. W. A. 1961. Carbohydrate translocation in raspberry and soybean. Plant Physiol. 36:820-824.

Burris, R. H. 1974. Biological nitrogen fixation, 1924-1974. Plant Physiol. 54:443-449.

Burris, R. H. 1942. Distribution of isotopic nitrogen in Azotobacter vinlandii. J. Biol. Chem. 143:509.

Burris, R. H. 1971. Fixation by free-living microorganisms; enzymology. pp. 105-152. In J. R. Postgate (ed.). The chemistry and biochemistry of nitrogen fixation. Plenum Press, N. Y.

Caldwell, B. E. 1969. Initial competition of root-nodule bacteria on soybeans in a field environment. Agron. J. 61:813-815.

Caldwell, B. E. and G. Vest. 1970. Effects of Rhizobium japonicum strains on soybean yields. Crop Sci. 10:19-21.

Caldwell, B. E. and D. F. Weber. 1970. Distribution of Rhizobium japonicum serogroups in soybean nodules as affected by planting dates. Agron. J. 62:12-14.

Carpenter, P. L. 1965. Immunology and Serology. W. B. Saunders Co., Philadelphia, Pp. 212-241.

Caviness, C. E. and F. D. Miner. 1962. Effects of stand reduction in soybeans simulating three-cornered alfalfa hopper injury. Agron. J. 54:300-302.

Damirgi, S. M., L. R. Frederick and J. C. Anderson. 1967. Serogroups of Rhizobium japonicum in soybean nodules as affected by soil types. Agron. J. 59:10-12.

Delwiche, C. C. 1978. Legumes--past, present, future. Bioscience. 28:565-570.

Delwiche, C. C., C. M. Johnson and H. M. Reisenauer. 1961. Influence of cobalt on nitrogen fixation by Medicago. Plant Physiol. 36:73.

DeMoody, C. J., J. Pesek and E. Spaldon. 1973. Mineral Nutrition. pp. 267-352. In B. E. Caldwell (ed.) Soybeans: improvement, production, and uses. Agron. Ser. 16 Amer. Soc. Agron., Inc., Madison, Wis.

Diatloff, A. 1965. Larvae of Rivellia (Diptera: Platystomatidae) attacking the root nodules of Glycine javanica. L. J. Entomol. Soc. Queensland. 4:86.

Dunphy, E. J. and J. J. Hanway. 1976. Water-soluble carbohydrate accumulation in soybean plants. Agron. J. 68:697-700.

Eastman, C. E. 1976. Infestation of root nodules of soybean by larvae of the bean leaf beetle, Cerotoma Trifurcata (Forster), and the Platystomatid fly, Rivellia quadrifasciate (Macquart). Ph.D. Thesis, Louisiana State University.

Erdman, L. W. 1943. New developments in legume inoculation. Soil Sci. Soc. Amer. Proc. 8:213-214.

Erdman, L. W. and F. S. Wilkins. 1928. Soybean inoculation studies. Iowa Agr. Exp. Sta. Res. Bull. 114:56p.

Evans, H. L. and S. A. Russell. 1971. Physiological chemistry of symbiotic nitrogen fixation by legumes. Pp. 191-244. In J. R. Postgate (ed.) The chemistry and biochemistry of nitrogen fixation. Plenum Press, N. Y.

Fehr, W. R., C. E. Caviness, D. T. Burmood and S. S. Pennington. 1971. Stage of development descriptions for soybeans (Glycine max L. (Merrill). Crop Sci. 11:929-931.

Garcia, R. L. and J. J. Hanway. 1976. Foliar fertilization of soybeans during the seed-filling period. Agron. J. 68-653-657.

Goodchild, D. J. and F. J. Bergerson. 1966. Electron microscopy of the infection and subsequent development of the soybean nodule cells. J. Bact. 92:204-213.

Gutschick, V. P. 1978. Energy and nitrogen fixation. Bioscience. 28:571-575.

Hallsworth, E. G., S. B. Wilson and E. A. N. Greenwood. 1960. Cobalt and copper in nitrogen fixation. Nature. 187:79-80.

Ham, G. E., V. B. Cardwell and H. W. Johnson. 1971. Evaluation of Rhizobium japonicum inoculants in soils containing naturalized populations of rhizobia. Agron. J. 63:301-303.

Hardy, R. W. F. 1977. Rate-limiting steps in biological photoproductivity. Pp. 369-400. In A. Hollaender (ed.) Genetic engineering for nitrogen fixation. Plenum Press, New York.

Hardy, R. W. F. and U. D. Havelka. 1974. The nitrogen barrier. Crops Soils. 26:10-13.

Hardy, R. W. F. and U. D. Havelka. 1975b. Nitrogen fixation research: a key to world food? Science. 188:633-643.

Hardy, R. W. F. and U. D. Havelka. 1975a. Pp. 421-439. In P. S. Nutman (ed) Symbiotic nitrogen fixation in plants. Cambridge University Press, London.

Hardy, R. W. F., R. D. Holsten, E. K. Jackson and R. C. Burns. 1968. The acetylene-ethylene assay for $N_2$ fixation: laboratory and field evaluation. Plant Physiol. 43:1185-1207.

Hewitt, E. J. 1958. Some aspects of mineral nutrition in legumes. Pp. 15-41. In E. G. Hallsworth (ed.). Nutrition of legumes. Butterworths, London.

Jackson, R. D. 1967. Soybean insect problems. Soybean Dig. 27:16-18.

Johnson, H. W., U. M. Means and C. R. Weber. 1965. Competition for nodule sites between strains of Rhizobium japonicum applied as inoculum and strains in the soil. Agron. J. 57:179-185.

Jordan, D. C. and E. H. Garrard. 1951. Studies on the legume root nodule bacteria. I. Detection of effective and ineffective strains. Can. J. Bot. 29:360-372.

Klucas, R. V., B. Koch, S. A. Russell and H. J. Evans, 1968. Purification and some properties of the nitrogenase from soybean (Glycine max Merr.) nodules. Plant Physiol. 43:1906-1912.

Lawn, R. J. and W. A. Brun. 1974. Symbiotic nitrogen fixation in soybeans. III. Effect of supplemental nitrogen and intervarietal grafting. Crop Sci. 14:22-25.

Lawn, R. J. and W. A. Brun. 1974b. Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source - sink manipulations. Crop Sci. 14:11-16.

Lehninger, A. L. 1970. Biochemistry. Worth Publishers, Inc., New York. Pp. 559-563.

Lie, T. A. 1974. Environmental effects on nodulation and symbiotic nitrogen fixation. Pp. 555-578. In A. Quispel (ed.). The biology of nitrogen fixation. Amer. Elsevier Publ. Co., Inc., New York.

Lindstrom, E. S., J. W. Newton and P. W. Wilson. 1952. The relationships between photosynthesis and nitrogen fixation. Proc. Nat. Acad. Sci., U. S. 38:392-396.

Lowther, W. L. and J. F. Loneragan. 1968. Calcium and nodulation of subterranean clover (Trifolium subterraneum L.). Plant Physiol. 43:1362-1366.

Manil, P. 1958. The legume - rhizobia symbiosis. Pp. 124-133. In E. G. Hallsworth (ed.). Nutrition of legumes. Butterworths, London.

McConnell, W. R. 1915. A unique type of insect injury. J. Econ. Entomol. 8:261-266.

McCoy, E. 1932. Infection by Bact. radicicola in relation to the microchemistry of the host's cell wall. Roy. Soc. (London), B. Proc. 110:514-533.

Means, U. M., H. W. Johnson and R. A. Date. 1964. Quick serological method of classifying strains of Rhizobium japonicum in nodules. J. Bacteriol. 87:547-553.

Metcalf, M. A. and W. P. Flint. 1951. Destructive and useful insects: their habits and control. McGraw-Hill Book Co., Inc., New York, Pp. 475-503.

Minchen, F. R. and J. S. Pate. 1973. The carbon balance of a legume and the functional economy of its root nodules. J. Exp. Bot. 24:259-271.

Namba, R. 1956. A revision of the flies of the genus Rivellia (Otitidae, Deptera) of America north of Mexico, U. S. Nat. Mus. 106:21-84.

Norman, A. G. and L. O. Krampitz. 1945. The nitrogen nutrition of soybeans. II. Effects of available soil nitrogen on growth and nitrogen fixation. Soil Sci. Soc. Amer. Proc. 10:191-196.

Nutman, P. S. 1958. The physiology of nodule formation. Pp. 87-107. In E. G. Hallsworth (ed.). Nutrition of legumes. Butterworths, London.

Nutman, P. S. 1965. Symbiotic nitrogen fixation. Pp. 363-379. In W. V. Bartholomew and F. E. Clark (eds.). Soil nitrogen. Agron. Ser. 10. Amer. Soc. Agron., Inc., Madison, Wis.

Nutman, P. S. 1976. Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge.

Parker, M. B. and H. B. Harris. 1977. Yield and leaf nitrogen of nodulating and nonnodulating soybeans as affected by nitrogen and molybdenum. Agron. J. 69:551-554.

Pate, J. S. 1962. Root - exudation studies on the exchange of $C^{14}$-labeled organic substances between the roots and shoot of the nodulated legume. Plant Soil. 17:333-356.

Pears, L. M. and R. H. Davidson. 1956. Insect pests of farm, garden and orchard. John Wiley and Sons, Inc., New York, pp. 228-272.

Pendleton, J. W. and E. E. Hartwig. 1973. Management. Pp. 211-237. In B. E. Caldwell (ed.). Soybeans: improvement, production and uses. Agron. Ser. 16. Amer. Soc. Agron., Inc., Madison, Wis.

Postgate, J. R. 1972. Biological nitrogen fixation. Merrow Publishing Co., Great Britain. Pp. 1-53.

Postgate, J. R. 1971. The chemistry and biochemistry of nitrogen fixation. Plenum Press, London, 326 p.

Sanders, J. L. and D. A. Brown. 1976. Effects of variations in the shoot:root ratio upon the chemical composition and growth of soybeans. Agron. J. 68:713-717.

Seegar, J. R. and M. E. Maldagne. 1960. Infestation de nodules de legumineuses en region equatoriale par des larves de Rivellia sp. (Dipt.). Parasitica 16:75-84.

Sloger, C. 1969. Symbiotic effectiveness and $N_2$ fixation in nodulated soybeans. Plant Physiol. 44:1666-1668.

Sloger, C. D., Bezdeicek, R. Milberg and Boonkerd. 1975. Seasonal and diurnal variations in $N_2$ ($C_2H_2$)-fixing activity in field soybeans. Pp. 271-284. In W. D. P. Stewart (ed.). Nitrogen fixation by free-living micro-organisms. IBP 6. Cambridge University Press.

Sprent, J. I. 1972. The effects of water stress on nitrogen fixing root nodules. IV. Effects on whole plants of Vicia faba and Glycine max. New Phytol. 711:603-611.

Stewart, W. D. P. 1966. Nitrogen fixation in plants. Athlone Press, Univ. of London, London, 168 p.

Tanner, J. W. and I. C. Anderson. 1964. External effects of combined nitrogen on nodulation. Plant Physiol. 39:1039-1043.

Tugwell, P. and F. D. Miner. 1967. Soybean injury caused by the three-cornered alfalfa hopper. Arkansas Farm Res. 16:12.

Turnipseed, S. G. 1973. Insects. Pp. 545-572. In B. E. Caldwell (ed.). Soybeans: improvements, production, and uses. Agron. Ser. 16. Amer. Soc. Agron., Madison, Wis.

Turnipseed, S. G. 1972. Response of soybeans to foliage losses in South Carolina. J. Econ. Entomol. 65:224-229.

Van Schreven, D. A. 1958. Some factors affecting the uptake of nitrogen by legumes. Pp. 137-163. In E. G. Hallsworth (ed.). Nutrition of legumes. Butterworths, London.

Vernon, L. P. and S. Aronoff. 1952. Metabolism of soybean leaves. Arch. Biochem. Biophys. 36:383-398.

Vest, G., D. F. Weber and C. Sloger. 1973. Nodulation and nitrogen fixation. Pp. 353-390. In B. E. Caldwell (ed.). Soybeans: improvement, production, and uses. Agron. Ser. 16. Amer. Soc. Agron., Inc., Madison, Wis.

Vorhees, J. H. 1915. Variations in soybean inoculation. J. Amer. Soc. Agron. 7:139-140.

Walters, H. J. 1964. Transmission of bean pod mottle virus by bean leaf beetles. Phytopathology 54:240.

Weber, C. R. 1955. Effects of defoliation and topping simulating hail injury to soybeans. Agron. J. 47:262-266.

Weber, C. R. 1966. Nodulating and nonnodulating soybean isolines: II. Response to applied nitrogen and modified soil conditions. Agron. J. 58:46-49.

Weber, D. F. and V. L. Miller. 1972. Effect of soil temperature on Rhizobium japonicum serogroup distribution in soybean nodules. Agron. J. 64:796-798.

Weil, R. R. and A. J. Ohlrogge. 1975. Seasonal development of, and the effect of interplant competition on, soybean nodules. Agron. J. 67:487-490.

Wilson, P. W. 1971. The background. Pp. 1-18. In J. R. Postgate (ed.). The chemistry and biochemistry of nitrogen fixation. Plenum Press, N. Y.

Wilson, P. W. 1940. The biochemistry of symbiotic nitrogen fixation. The University of Wisconsin Press, Madison. 302 p.

Winter, H. C. and R. H. Burris. 1968. Stoichiometry of the adenosine triphosphate requirement for $N_2$ fixation and $H_2$ evolution by a partially purified preparation of Clostridium pasteurianum. J. Biol. Chem. 243:940.

Wright, W. H. 1925. The nodule bacteria of soybeans. I. Bacteriology of strains. Soil Sci. 20:95-129.

VITA

Robert L. Hutchinson, son of Jesse M. and Hildur A. Hutchinson, was born July 4, 1951, at Alexandria, Louisiana. He attended public schools at Pineville and Tioga, Louisiana, and graduated from Tioga High School in 1969.

He received a B. S. degree in Biology from Northwestern State University, Natchitoches, Louisiana, in 1974, and a M. S. degree in Agronomy from Louisiana State University in 1976. At present he is a candidate for a Ph.D. in the Department of Agronomy at LSU.

He is a member of Gamma Sigma Delta, Sigma Xi, American Society of Agronomy and Soil Science Society of America.

**EXAMINATION AND THESIS REPORT**

Candidate: Robert L. Hutchinson

Major Field: Agronomy

Title of Thesis: The effects of insect damage on $N_2$ fixation by soybeans and recovery of Rhizobium japonicum, strain 110, from soybean nodules.

Approved:

Edward P. Dunigan

Major Professor and Chairman

James G. Traynham

Dean of the Graduate School

EXAMINING COMMITTEE:

W. H. Willis

L. D. Newsom

Jeme P. Hernandez

Joe E. Sedberry, Jr.

D. L. Robinson

Date of Examination:

February 14, 1979